Nitrates in a catchment cleared of alien woody legumes in relation to ground water quality in the Atlantis Aquifer (South Africa)

Nicolette van der Merwe

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Supervisor: Dr A. Rozanov

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Declaration

I declare that this research is my own and that it was conducted under the supervision of Dr A. Rozanov. No part of this research has been submitted in the past, or is being submitted for a degree at another university.

Signature: _____

Nicolette Van der Merwe

Date: _____/_____/_____

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Abstract

The sandy soils of the Riverlands Nature Reserve, near Malmesbury, and the neighbouring farm were studied to determine the effects of long term invasion of the legume *Acacia saligna* on the soil nutrient content of a soil previously vegetated with fynbos. The effect of the removal of this alien legume on general soil properties and groundwater quality were also studied. The changes in nitrates and nitrites (NO_X) due to the invasion and removal of the alien legume were investigated in more detail than changes in other soil nutrients. In addition to that emphasis was placed on the effect of vegetation clearing on groundwater quality, specifically relating to potential contamination with nitrates.

This study was initiated after Conrad et al., (1999) found increased NO_X concentrations in ground water while studying the effects of pig farming on ground water nitrogen (N) near a site cleared of *Acacia saligna* by Working for Water (WFW). Since many sites are scheduled for removal of this alien vegetation it was deemed necessary to study the effects that clearing alone had on groundwater quality. It was suspected that there would be an increase in soil and groundwater NO_X with vegetation removal due to the inputs from the legume alien invader.

Soil sampling was done continuously throughout the rainy season of 2007 (From May to December) on three adjacent sites separated by some 50 m of distance, consisting of a natural fynbos site and two *Acacia saligna* sites. The sites were selected approximately on the same contour line to prevent interaction through lateral water flow. One of the Acacia sites was cleared by the Working for Water programme in the usual manner leaving slash on the ground. Soil samples were collected at regular intervals throughout the season from all three sites (fynbos, Acacia and cleared site) using a Jarrett soil auger. They were airdried (to achieve full oxidation of mineral N) and sieved though a 2mm sieve. Soils were analysed by atomic absorption spectroscopy for basic cations and by ion chromatography for anions, including nitrates and nitrites. Total carbon and nitrogen was determined by combustion, pH (1M KCl and H_2O) and EC (1:5 H_2O) were also

measured. The present study was part of a wider investigation into the quality of groundwater, modelling flow and contaminant transport (Jovanovic et al., 2008).

The largest changes in soil properties were observed in the top (0-5 cm) layer. The fynbos site had a lower nutrient status by comparison to the Acacia site and the cleared site. The sum of cations from the soils of the fynbos site at a soil depth of 0-5cm was 100mg/kg and soils from the cleared and Acacia sites were about 190mg/kg. The Acacia site had a higher NO_X status and experienced a larger NO_X fluctuation during the rainy season than the fynbos site; minimum NO_X values were similar (below 10mg/kg) but the Acacia site had a maximum NO_X value of near 60mg/kg and the fynbos site just below 30mg/kg. There was little difference in general soil characteristics (exchangeable cations, pH and EC, total soil C and N) during the first season after clearing, between the Acacia and the cleared sites.

The effect of soluble nitrogen changes due to alien legume invasion and removal on groundwater quality, relating to NO_X, during the first season after clearing, was determined. It was found that the Acacia site had higher NO_X concentrations than the fynbos site. At 0-5cm the fynbos site NO_X was less than 30mg/kg and the Acacia site was between 30 and 110mg/kg for most of the season, with values lower than 30mg/kg for the last four sampling dates only. N concentrations on the cleared site behaved in a similar manner to the uncleared Acacia site, but generally N values were lower on the cleared site, there were only two sampling dates where the cleared site had higher NO_X values than the Acacia site at 0-5cm. The average groundwater N in NO_X under the cleared site was 4.34 mg/l, and under the Acacia site 3.78mg/l, these values are both below the level determined for water contamination with nitrates. However, the increase in ground water nitrate levels after *A. saligna* clearing was significant.

It was concluded that there is a change in the nutrient status of soil with Acacia invasion and again with removal. NO_X migrates to the groundwater to a larger degree once

vegetation has been removed, although during this study the nitrate pollution threshold of 10 mg/l nitrate N was not reached.

Opsomming

Die sanderige gronde van die Riverlands Natuur Reservaat, naby Malmesbury, en die aangrensende plaas was bestudeer om die effek van die langtermyn indringing van die peulgewas, *Acacia saligna*, op die voedingswaarde van ‘n voorheen fynbos begroeide grond, sowel as die effek van die verwydering van die indringer op die algemene samestelling van die grond en grondwater kwaliteit. Oplosbare stikstof veranderings (NO_X) wat plaasvind as gevolg van die indringing en verwydering van die indringer peulgewas, was in meer diepte bestudeer as die ander elemente. Klem was ook geplaas op die effek van die verwydering van plantegroei op grondwater kwaliteit, met spesifieke verwysing na potensiële nitraat besoedeling.

Die studie was beplan na Conrad et al., (1999) ‘n toename van NO_X konsentrasies in grondwater ontdek het, tydens ‘n studie van die effek wat vark boerdery het op grondwater N naby ‘n area waar *Acacia Saligna* verwyder was deur Working For Water (WFW). As gevolg van die feit dat verskeie areas in die proses is om skoongemaak te word van *Acacia Saligna,* is dit nodig geag om die effek daarvan of grondwater kwaliteit te ondersoek. Die hipotesis was dat daar ‘n toename in NO_X konsentrasies in grond en grondwater sal wees as gevolg van die verwydering van plantegroei.

Grond monsters was deurlopend geneem gedurende die reën seisoen van 2007 (Vanaf Mei tot Desember) uit nabygeleë areas wat omtrent 50 meter uitmekaar is. Die volgende tipes grond was verteenwoordig: ‘n Fynbos begroeide grond sowel as ‘n grond begroei met *Acacia saligna*. Die areas was geselekteer ongeveer op die selfde kontoer lyn om interaksie tussen areas te voorkom as gevolg van laterale water vloei. ’n Gedeelte van die *A. saligna* area was skoongemaak deur die Working for Water program op die gewone manier deur die afgesnyde plant materiaal op die grond te los. Grond monster was geneem met gereelde intervalle gedurende die seisoen op al drie areas (fynbos, *A.*

saligna, en skoongemaakte area) deur die gebruik van ’n Jarret grondboor. Die monsters was lugdroog gemaak (om volle oksidasie van die mineraal N te verseker) en toe gesif deur ‘n 2mm sif. Die gronde was analiseer deur atoom absorpsie vir basiese katione en deur ioon chromatografie vir anione, insluitende nitriete en nitrate. Totale koolstof en stikstof was bepaal deur verbranding terwyl pH (1M KCl en H_2O) en EC (1:5 H_2O) ook gemeet was. Hierdie studie was deel van ‘n wyer ondersoek na die kwaliteit van grondwater, vloei modelering en vervoer van kontaminante (Jovanovic et al., 2008).

Die grootste veranderinge in die grond eienskappe was in die boonste grondlaag (0-5cm) waargeneem. Die bevinding was dat die fynbos area ‘n laer voedingswaarde het as die area begroei met die indringer sowel as die die skoongemaakte area. Die som van katione onder fynbos grond by ‘n grond diepte van 0-5cm, was 100/mg/kg en die som van katione by die ander twee areas was omtrent 190mg/kg. Die Acacia area het ‘n hoër NO_X inhoud en het ‘n groter NO_X fluktuasie ervaar gedurende die reën seisoen as die fynbos area. Die minimum NO_X waardes was soortgelyk (minder as 10/mg/kg), maar die Acacia area het ‘n maksimum NO_X waarde van omtrent 60mg/kg terwyl die fynbos area se maksimum net minder as 30mg/kg gehad het. Daar nie veel verskil tussen die algemene grond eienskappe (uitruilbare katione, pH en EC, totale C en N) van die Acacia en skoongemaakte areas gedurende die eerste seisoen na die indringer verwyder is nie.

Die effek van oplosbare stikstof veranderings as gevolg van die peulgewas se indringing en verwydering op grondwater kwaliteit, met verwysing na NO_X, gedurende die eerste seisoen na verwydering van die indringer was bepaal. Dit was bevind dat die Acacia area ‘n hoër NO_X konsentrasie as die fynbos area het. Op ‘n diepte van 0-5cm was die fynbos NO_X laer as 30mg/kg terwyl die Acacia area tussen 30 en 110mg/kg vir die grootste gedeelte van die seisoen was met waardes laer as 30mg/kg vir die laaste vier datums waarop monsters geneem was. Veranderings in N op die skoongemaakte area en die Acacia area was soortgelyk, maar oor die algemeen was N waardes laer op die skoongemaakte area. Daar was slegs op twee datums gevalle gevind waar die skoongemaakte area ‘n hoër NO_X inhoud as die Acacia area gehad het by ‘n diepte van 0-5cm. Die gemiddelde N in NO_X in die grondwater onder die skoongemaakte area was

4.34mg/l en by die Acacia area was dit 3.78mg/l; hierdie waardes is onder die drempel vir nitraatbesoedeling in grondwater. Die nitraat inhoud van die grondwater het wel beduidend toegeneem as gevolg van verwydering van *A. saligna*.

Die gevolgtrekking was dat daar ‘n verandering plaasvind in die voeding status van grond met *Acacia saligna* indringing en weer met verwydering. NO_X migrasie na die grondwater verhoog met verwydering van plantegroei. In hierdie studie was die nitraat besoedeling drempel van 10mg/l nitraat N nie bereik nie.

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Chapter 1: Introduction

Acacia saligna (commonly known as Port Jackson or Port Jackson Willow) is the leading alien invader in the Western Cape with regards to both the area of invasion and potential future invasion (Richardson et al., 1992). Invasion by Acacia saligna has been seen to be a threat to biodiversity and the ecosystem, but little is known about the effect on nutrient cycling in the soil (Ehrenveld, 2003). Knowledge of N accumulation due to the cultivation of nutrient poor soils and changes due to alien invasive plants is necessary in order to determine the viability and potential success of restoration of natural fynbos vegetation. The changes in N dynamics due to the invasion and subsequent clearing of this leguminous alien invader will affect the restoration process. Therefore, the N and other nutrient changes due to leguminous alien invasion and subsequent removal must be investigated. The potential for groundwater pollution as a result of rainfall after vegetation removal and other causes of N additions on these sandy soils should also be assessed.

This study was initiated by the Water Research Committee of South Africa as a follow up to the findings of Conrad et al., (1999) who, while studying the effects of pig farming on N leaching into ground waters, have observed an increase in groundwater NO_X (nitrates and nitrites) concentrations upon removal of the alien vegetation upstream by the Working for Water team.

It was found that N in groundwater increased, but since the catchment in which alien acacias were cleared was not sampled or monitored specifically, it was deemed necessary to determine, in an independent study, whether or not the increases in groundwater NO_X may happen as a result of vegetation clearing.

Invasion of the Acacia results in a change in nutrient cycling, with higher N and P contents in litter under Acacia vegetation (Macdonald and Richardson, 1986) and larger amounts of above-ground mass as well as changes in fire-regimes and hydrology (Versfeld and Van Wilgen, 1986). Biodiversity is also affected and a decrease in species

richness and vertebrate diversity has been documented (Macdonald and Richardson, 1986; Van Wilgen and Richardson, 1985).

This issue is of ultimate importance since Working for Water is a wide-scale programme ultimately aiming at removing dense growth of *Acacia saligna* throughout the Cape Flats area. The area of invasion in the Swartland currently occupies some 232 556ha (Versfeld et al., 1998) and vegetation clearing on such a grand scale may result in wide-spread nitrate pollution of the strategically important shallow aquifer, which supplies both drinking and irrigation water to the local population.

The present study was part of a wider investigation into the quality of groundwater, modelling flow and contaminant transport (Jovanovic et al., 2008). Fields of study involved were soil physics, hydrology, hydrogeology, hydrogeochemistry and a brief view of weather data and plant root distribution. All these results made up a data set used for modelling NO_X seepage from the soil surface down to the ground water table through the vadose zone.

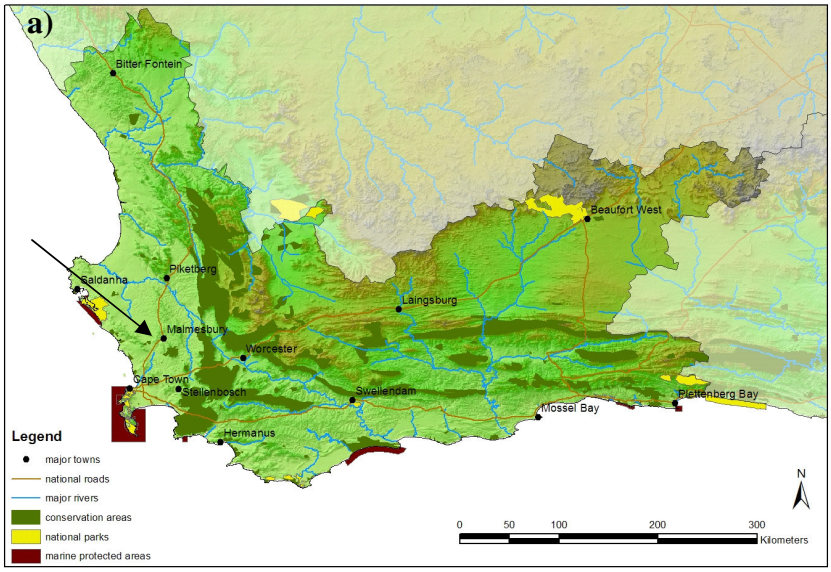
Groundwater was studied in detail with measurement of all major nutrients, but specifically soluble N. The soil data gained from this study was used with the groundwater data and some weather data to develop a model. The aim of the model was to predict whether groundwater pollution has taken place and could still take place due to the removal of the alien vegetation.

The objectives were:

1. Study the soil properties of adjacent sites under fynbos and *Acacia saligna* to understand the differences in nutrient status, particularly nitrogen
2. Observe the effect of *Acacia saligna* clearing on changes in NO_X concentrations with depth in the soil, in relation to the natural fynbos vegetation
3. Investigate the potential for groundwater pollution after clearing of the invasive alien legume *Acacia saligna*

Figure 1-1 is a map of the location and figures 1-2 and 1-3 are photographs taken of the sites selected for this study.

To carry out these objectives three experimental sites, as designed by the CSIR, WFW and the Department of Water Affairs and Forestry (DWAF), representing three vegetation types were studied for nutrient contents over one wet season, specifically with relation to nitrogen dynamics. Chapter two is a review of current literature on the subjects of nitrogen dynamics as well as the different vegetation systems in relation to nitrogen, and leaching of soluble nitrogen. Changes in soil properties due to alien legume (*Acacia saligna*) invasion and clearing is reported in chapter three and chapter four reports the impacts of this clearing on nitrogen in soil and groundwater.



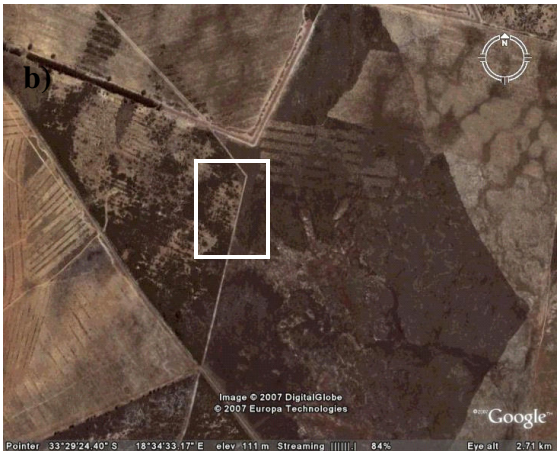


Figure 1-1: a) Placement of the Riverlands Nature Reserve in the Western Cape

Figure 1-2: Photo of the fynbos study site on the Riverlands Nature Reserve

Figure 1-3: Photo of the Acacia site on the Burgerpost farm neighbouring Riverlands Nature Reserve

Chapter 2: Nitrogen dynamics in soil and the effects of clearing vegetation on soil N and water quality – A Review

2.1 Introduction

Almost 90% of total N is found in soil or in organic form, as components of litter and living organisms (Martin and Plassard, 2001). Less than 1% of N is in inorganic form and available to plants (Martin and Plassard, 2001). Considering the small amount of N available to plants, it is important to consider N-dynamics in conjunction with the N demand of the natural vegetation in the particular area (Coessens et al., 1999). Since N is often limiting in ecosystems - terrestrial and aquatic - legumes are often used to increase soil fertility, in particular soil N (Vitousek et al., 2002; Postgate, 1978), because legumes have the unique ability to enrich the soil with N through symbiotic relationships with soil organisms (Burns and Hardy, 1975; Barea et al., 1988). In the short term total soil N is a fairly constant factor, but due to it being involved in dynamic processes and systems, it can vary over longer periods of time (Du Preez, 1987).

Areas with natural fynbos tend to have a low soil nutrient status (Lamb and Klaussner, 1988; Stock and Allsopp, 1992). They are often dystrophic and strongly leached (Specht and Moll, 1983), because of the lack of buffering, they render the groundwater susceptible to contamination (Neeteson, 1999). Due to the shortage of water in South Africa, water resources need to be protected (Momba et al., 2006) and the potential for N pollution taken into account.

Being a legume, the growth of *Acacia saligna* on a site will tend to increase the total soil N and potentially increase the total organic matter (and subsequently the soil N content) because of its higher litter inputs on soils low in N (Yelenik et al., 2007). Acacia also have the ability to redistribute N and other major plant nutrients through the topsoil and in so doing increase their availability (Lamb and Klaussner, 1988).

The purpose of this review is to study the current knowledge of nitrogen dynamics in soils as well as the effects of leguminous alien invasion and subsequent clearing on soil nutrients and groundwater nitrates. This is done in order to understand the gaps in current knowledge and assist in the understanding of experimental findings.

2.2. N Dynamics in soil

Rainfall and temperature are the dominating soil forming factors responsible for spatial variation of N in soils (Day et al, 1978). This is because they determine the rate of N-conversions and the occurrence of anaerobic microenvironments (Day et al., 1978; Olivares et al., 1988; Biggar, 1978). Under trees the spatial variation will usually tend to be skewed, this is due to the non-uniform distribution of trees in nature and the localised temperature and sunlight gradient (Biggar, 1978). N pathways are both transient and dynamic, with processes leading to both gains and losses of N in various forms. Figure 2-1 is a summary of the N processes within the soil.

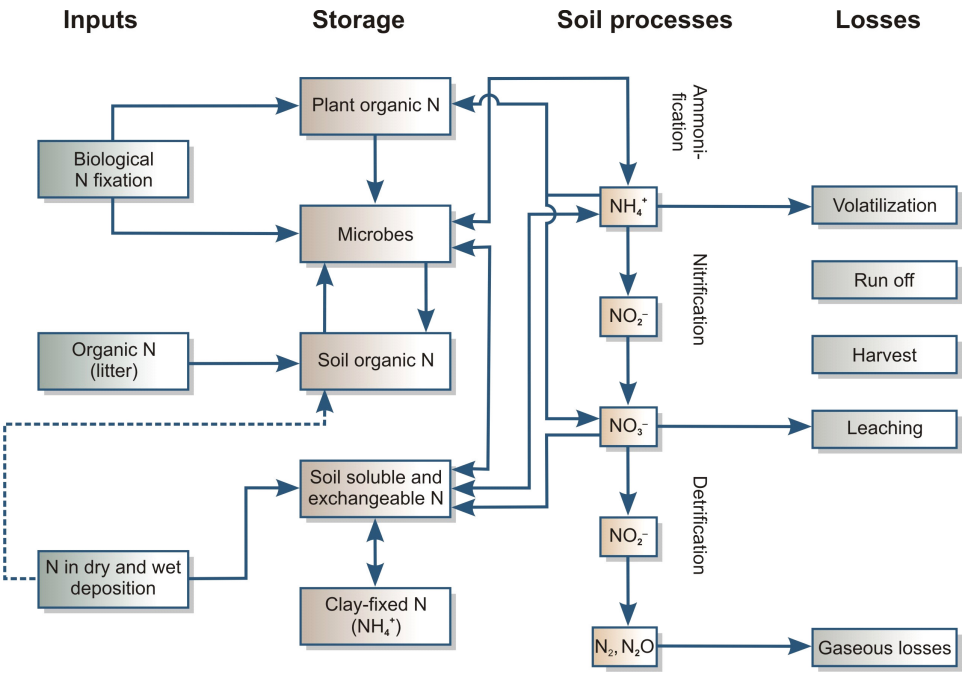


Figure 2-1: Interactions of N processes in soils (adapted from Rosswall, 1976)

2.2.1 Gains of nitrogen in soils

2.2.1.1. N fixation

By definition, N fixation is "The conversion of elemental N to organic forms or forms readily used in biological processes" (Van der Watt and Van Rooyen, 1995). The process of N fixation is limited to bacteria (Postgate, 1978). NH_3 is the first product in the two step process of N_2 reduction as N fixation by bacteria (see Equation 2-1) and is synonymous with the Haber-bosch process which is used for the industrial production of N fertiliser. The final product in this process, NH_4^+ is now an available form of N for plant use (Kammen, 1997).

$$N_2 + 3H_2 \xrightarrow{\text{Bacteria}} 2NH_3 + H_2O \xrightarrow{\text{Bacteria}} 2NH_4^+ + \tfrac{1}{2}O_2$$

Equation 2-1: The chemical pathways of transforming N_2 to NH_4^+ (Postgate, 1978)

Nitrogen fixing bacteria are responsible for over 70% of the N in soil and water (Postgate, 1978) making N fixation the major contributor to N additions in the soil (Cocks and Stock, 2001). To fertilise the soil with the same amount of N as NH_4^+ that has been biologically fixed, utilising the Haber-Bosch process would require about 188Tg of energy and cost 30 billion dollars per year, and this was in 1997 (Vance, 1997) with inflation rates as high as 13% year on year that would be hundreds of millions of rand in this day and age. Crop rotation with legumes has been used to decrease the need for N fertilising ever since crop production began, this is a good alternative, both environmentally suitable and economically viable (Vance, 1997).

The energy supply for the process of N fixation is from the sun, and this energy can be used directly by the micro-organism or via the plant by conversion to carbon compounds (Postgate, 1978). These carbon compounds are then gained by the microbe through the shared nodule in a plant specific symbiotic plant-*Rhizobium* relationship (Postgate, 1978). About 80% of total biological N fixation that takes place in soil is due to the symbiosis between *Rhizobium* and legumes through root nodules (Kammen, 1997). This is the most

important symbiotic relationship for N fixation and has been well studied (Vitousek et al., 2002).

Symbiotic bacteria have the ability to reduce atmospheric N_2 to NH_4^+, making it available to the plant (Day et al., 1978). This symbiotic relationship occurs through a shared organ, the nodule that is established for the symbiotic relationship between N-fixing bacteria and plants, primarily legumes (Trinchant et al, 2001) These nodules are only active for a couple weeks (Trinchant et al, 2001). The bacteria *Rhizobium*, can reduce atmospheric N_2 in the soil to NH_4^+ for use by the plant, in exchange carbon sources of energy are made available to the bacteria by the plant (Trinchant et al, 2001). This ability to fix N is enormous, it can be more than 100kg/ha per year (Vitousek et al., 2002). This relationship need not be symbiotic; some bacteria improve the N availability of the root zone without penetrating the nodule (Trinchant et al, 2001).

A symbiotic relationship with mycorrhizae is also beneficial to plants. This relationship can improve the ability of the plant to absorb mineral N and occurs most commonly with forest trees (Trinchant et al, 2001). NH_4^+ and NO_3^- are the most common forms of N absorbed by plants from the soil, to a lesser degree amino acids and urea are also absorbed (Martin and Plassard, 2001). Mycorrhizae can facilitate the uptake of organic forms of N (amino acids, other proteins, peptides) from soil by the plant, without this interaction, these forms of N are poorly utilized by higher plants (Martin and Plassard, 2001). Even so, where mycorrhizae are abundant, NH_4^+ remains the most common form of N uptake from soil (Martin and Plassard, 2001). Mycorrhizae can also facilitate N transfer between plants (Van Kessel et al., 1985). In pot trials done by Hoffman and Mitchell (1986) on *A. saligna* mycorrhizal density decreased with increased seedling density, it was suggested that this was due to competition for limited nutrients.

There are factors that can limit the ability of N-fixation by symbiosis with the result that *N-fixers* will be at a greater disadvantage than non N-fixers in a particular environment (Vitousek et al., 2002, Vitousek and Field 1999). N-fixation is a process requiring high energy levels; this can be a potential limitation due to lack of energy resources (Barea et

al., 1988). Potential restraints when compared to plant growth of non N-fixers include specific oxygen levels for the activation of nitrogenase enzymes. Many of these enzymes also require Mo, P and Fe, excess combined N can impede N-fixation (Vitousek et al., 2002; Barea et al., 1988). Further limitations include the lack of shade tolerance (Vitousek and Howarth, 1991) and grazing of protein- rich tissues and plants (Ritchie and Tilman, 1995).

2.2.1.2. Atmospheric Deposition

N can be added to the soil through dry deposition through plants by transpiration, or wet deposition by precipitation of gas and particle matter (De Wever et al., 1999; Söderlund, 1981). N compounds for dry deposition can be associated with atmospheric particles, these can vary in size depending on the form of N (Söderlund, 1981). Little is know about the dry deposition of N with gasses, and dry deposition has mainly been studied for sulphur dioxide and ozone. The solubility of N is affected by pH and this will affect deposition to wet surfaces. Assimilation of N into water particles for wet deposition can occur in the clouds or below them, atmospheric mixing is complex and the origin of N by wet deposition is difficult to ascertain (Söderlund, 1981). Most N deposition occurs in industrialised areas. Main sources of atmospheric N are from NO created by fossil fuel combustion and livestock (Söderlund, 1981; Söderlund and Svensson, 1976).

In studies done by Wilson et al., 2008 the nitrogen content of plants (moss tissue) and soils studied showed and increase between the period from before 1940 to 1950-70 in the Cape Metropolitan area. The increase in N between samples from 1970 and those taken after 2000 was significantly larger. This is assumed to be an indication of atmospheric deposition given the close relationship N in the atmosphere and N in the tissue of moss (Baddely et al., 1994)

2.2.1.3. Plant residues and microbial decomposition

The decay of plant material and other organic litter contributes to the content of soil N (Otto et al., 1999). The autolysis of organic matter with the release of NH_4^+, known as

ammonification (Van der Watt and Van Rooyen, 1995), is catalysed by deaminase enzymes. This organic matter breakdown releases N in the form of free NH_3 into the environment (Postgate, 1978). Therefore the removal of plants will reduce the N in the system, although the remaining plant parts and organic litter will contribute to the organic N pool (Pratt et al., 1978). The release of nitrogen from litter is affected by decomposition and mineralisation (Berg and Staaf, 1981).

In studies done by Versveld and Van Wilgen (1986), fynbos was found to have very low amounts of litter, ranging from 0.78t/ha per year for 9 year old arid fynbos and 2.17t/ha per year for mesic fynbos of 21 years. This litter decomposes at a slow rate (Mitchell et al., 1986).

Berg and Staaf, (1981) have identified three basic phases of N changes during litter decomposition. The first of these is the leaching phase; this is short and involves a rapid release of N in leachable form. This is followed by the accumulation phase, in the case where litter is high in N this phase may not occur. This phase can be identified by an increase in soil N either after leaching or at the beginning of decomposition. The third phase is termed the "final release phase" and involves the release of N, either after rapid leaching or following accumulation. This phase may be indistinguishable from the leaching phase if accumulation does not occur. Typically it is characterised by a slower release of N than the leaching phase (Berg and Staaf, 1981).

2.2.1.4. Fertilizer application

Due to the importance of N as an essential element for competitive plant growth and the production of crops, it is the most widely used fertilizer comes at an enormous cost as previously discussed, and has had a hand in increasing agricultural production (Di and Cameron, 2002; Otto et al., 1999; Kammen, 1997; Vance, 1997). The Food and Agricultural Organisation (FAO) (2004) predict a worldwide increase in demand for N fertilisers of 1% per year until 2008/9, this is an increase of 4.7 million Tonnes worldwide, the growth in Africa being 3%. Africa has had a 10.4% increase in N fertiliser consumption since 2002 (FAO, 2004). Over- application and the excessive use of N

fertilisers is common and can have detrimental environmental effects (Otto et al., 1999; Kammen, 1997). An increase in the amount of N applied to the soil increases the rates of nitrification and denitrification and subsequently increases the amount of N_2O and N_2 losses as well as an increase in NO (Boeckx et al., 1999; Otto et al., 1999, Du Preez, 1987).

2.2.2 Nitrogen losses from soil

A loss of N from the soil can occur from the volatilisation of NH_4^+, denitrification and leaching of NO_3^- from the profile as well as by plant uptake (Agenbag and Vlassak, 1999). Other less common losses of available N from the soil are by chemical fixation of NH_4^+ to micaceous clay surfaces and, soil erosion (Pratt et al., 1978).

2.2.2.1 Volatilisation of ammonia

Volatilisation of ammonia, defined as the "loss of NH_4^+ from soil due to high concentrations in alkaline soils" by Van der Watt and Van Rooyen (1995), is responsible for a high degree of acidification of soil (Van Cleemput and Boeckx, 1999). Under most circumstances NH_4^+ is retained by soil (Haitt, 1978; Postgate, 1978). Volatilisation (Stock and Lewis, 1986) occurs due to surface application of N fertilizers. Thus careful management is therefore required to minimise loss in this manner.

2.2.2.2. Denitrification processes in soil

Denitrification results in the release of N either as N_2 or as an N oxide by the reduction of NO_3^- or NO_2^-. These gasses both have negative effects on the ozone layer and contribute to the greenhouse effect (Van der Watt and Van Rooyen, 1995; Van Cleemput and Boeckx, 1999). NO_3^- reduction takes place with the oxidation of organic matter, NO_3^- can be reduced to N_2 gas by bacteria, and these bacteria are found in nutrient rich soils and compost heaps (Postgate, 1978). Denitrification as a chemical and microbial reduction process is illustrated in figure 2-2 (Boeckx et al., 1999).

$NO_3^- \rightarrow N_2O \rightarrow N_2$

Figure 2-2: Denitrification reaction Boeckx et al, 1999

The chemical process of denitrification is usually as the result of accumulation of NO_2^- after over-application of fertilisers of ammonium or urea (White, 1997). Denitrification can alternativels, and more commonly, take place as a biological process under anaerobic soil conditions. For this process adequate supplies of organic substrate are required for the growth of microbial organisms (White, 1997).

2.2.2.3. Leaching of soluble N

Both NO_3^- and NO_2^- can act as groundwater pollutants, the degree of leaching of these products is a fine balance between different factors of the soil, environment (particularly rainfall) and management practices (Van Cleemput and Boeckx, 1999, Otto et al., 1999). In the case of NO_3^- leaching and water pollution, the depth of the water table and precipitation are influential factors in determining the degree of or potential for water pollution (Reitz, 1978).

After the harvesting of crops has taken place, N often remains in the soil, either as mineral residue or as organic matter (Neeteson, 1999). The mineralisation of these sources of N in the soil can contribute to NO_3^- leaching and the contamination of groundwater (Neeteson, 1999). The assimilation of NO_3^- into plants reduces the loss of NO_3^- by leaching (Du Preez, 1987) and decreases the N residues left in soil after harvesting. Leaching is one of the main causes of N loss from soil systems and should the nitrates leached reach groundwater; the environmental consequences can be detrimental due to the increase of base status of water bodies (Du Preez, 1987).

Reports of NO_3^- toxicity in humans have been mainly due to drinking NO_3^- rich well water with values higher than 10ppm, but also from eating vegetables (most commonly spinach) with high NO_3^- contents (Lorenz, 1978;). Monitoring and managing NO_3^- levels in well water and irrigation water is important as high levels negatively affect humans, animals and the environment (Lorenz, 1978).

2.2.3. Soil processes involving N

In an experiment done by Agrella et al, (1999) it was determined that rainfall patterns had the largest influence on mineralisation and nitrification rates, the reason for this was because of the stimulation of microbial activity in soil. When rainfall increases such that microcosms of anaerobic conditions are formed, denitrification with NO and N_2 production could also occur (Agrella et al., 1999, Greenwood, 1978). This is because the NO_3^- that was produced under aerobic conditions can diffuse to anaerobic microsites and become reduced (Greenwood, 1978). In general the soil solution NO_3^- is usually similar in magnitude to that of NH_4^+, however NH_4^+ can be present in concentations of up to ten times more than NO_3^- (Agrella et al., 1999). Mineralisation, N immobilisation and nitrification will be addressed as the main N processes in the soil. Ion interaction and the effect of this on plant uptake will also be considered.

2.2.3.1. N Mineralisation

The conversion of organic to inorganic forms of N with the help of micro-organisms is known as mineralisation (Agenbag and Vlassak, 1999). Ammonification is specifically the release of NH_4^+ from organic N (Van der Watt and Van Rooyen, 1995). Ammonium contributes to the total N available to plants (Otto et al., 1999). Reaction rates for ammonification and mineralisation are dependant on the soil moisture content and rainfall (Otto et al., 1999, De Wever et al., 1999). Coessens et al., (1999) adds some important factors influencing the mineralisation rate of a soil, these are, C: N ratio, microbial activity and composition of organic matter for decomposition.

Mineralisation takes place under slightly alkaline pH, at an optimum temperature of 30°C. Stock and Lewis, (1986) found that fires can act as a mineralising agent. The first products of mineralisation are NO_2 and hydronium, NO_2 is further oxidised by *Nitrobacter* at pH less than 9 to NO_3^- (Coessens et al., 1999; Day et al., 1978). This reaction can be reversed, with a supply of soluble organic material under anaerobic conditions, NO_3^- can be reduced to NO_2 (Day et al., 1978). This form of N can be toxic to mammals, even in small doses, but can be reduced by one of many denitrifying micro

organisms to N_2O or N_2. With a decrease in redox potential, there is a decrease in the ratio of N_2O to N_2 (Day et al., 1978).

Although NH_4^+ does not leach readily because it is adsorbed on soil colloids, leaching of NH_4^+ can occur in soils with low CEC. As well as this, nitrification with the production of leachable NO_3^- takes place easily. (Haitt, 1978; Postgate, 1978)

2.2.3.2. Nitrification

The oxidation of NH_4^+ by microbial activity to NO_2 and NO_3^- (Van der Watt and Van Rooyen, 1995) displayed by Figure 2-3.

.

$NH_4^+ \rightarrow N_2O(g) + NO_2 \rightarrow NO_3^-$

Figure 2-4 the nitrification reaction (Boeckx et al., 1999)

In aerobic environments, the nitrification reaction is dominant to the denitrification reaction; however the ratio between them varies (Greenwood, 1978). The soil properties and microbial community also play a determining role in the final ratio (Boeckx et al., 1999), Nitrification takes place in smaller amounts in acidic soils (Morot-Gaudry and Tourraine, 2001). Certain bacteria (*Nitrosomonas* and *Nitrobacter*, both photo- and chemo-autotrophs) can oxidize NH_4^+ to NO_3^- via NO_2 and are responsible for the process of nitrification (Postgate, 1978).

2.2.3.3. N Immobilisation

Immobilisation is the assimilation of N into organic matter (plant or microbial) from inorganic N (De Wever et al., 1999; Van der Watt and Van Rooyen, 1995). With an increase in organic matter the degree of immobilisation that takes place is increased (Coessens et al., 1999). Leaching can also be prevented by the sorption of nitrates onto inorganic particles such as iron and aluminim hydroxides (Ndala et al., 2006). positively

charged soil surfaces such as kaolinite can also increase the nitrate retention of a soil (Ndala et al., 2006) In this way pH can have an effect on the ability of a soil to retain nitrate, it is expected that at lower pH's nitrate retention will be higher that at higher pH's. This has been confirmed by Kinjo and Pratt (1971), but was not found by Ndala et al., (2006).

2.2.3.4 Storage effects on N mineralisation

Microbial population is decreased during prolonged storage as well as with drying. Drying also causes changes in N mineralisation to occur (Sparling and Cheshire, 1978; Nordmeyer and Richter, 1985). Nordmeyer and Richter, (1985) found that net mineralisation increases with storage, but with incubation periods of more than 30 days, the increase in net mineralisation is lower than for shorter periods of storage (Nordmeyer and Richter, 1985). The resistant, or slowly decomposable organic N was found to be mineralised with more difficulty in more sandy soils than in clay soils, and overall the potential for mineralisation increases with clay content (Nordmeyer and Richter, 1985). Mineralisation studies on disturbed soil samples differ from mineralisation in undisturbed soils. On disturbed soils there is a flush in mineralisation during the first 20 days of incubation, on undisturbed soils mineralisation that takes place is nearly linear (Nordmeyer and Richter, 1985). It is important to realise from this that disturbance of soil will alter the mineralisation, but that with increased periods of storage, mineralised N will better reflect what would occur in an undisturbed sample (Nordmeyer and Richter, 1985). This is because as mineralisation takes place, the soil N nears a state of full oxidation, this will account for the smaller differences in mineralised N after longer periods of incubation (Day et al., 1978).

2.2.3.5. Ion interactions and plant uptake

Experiments done by Rao and Rains (1976) showed that, although small, there was an effect on the uptake of NO_3^- by barley seeds in the presence of Cl^{2-}, SO_4^{2-} and Br^{2-} in the fertilising solution. The uptake of NO_3^- is therefore specific, and is influenced by other ions but only to a small degree (Rao and Rains, 1976).

Cations in solution did have significant effects on the uptake of NO_3^- in studies done by Huffaker and Rains (1978) but the effect varied with the cation, Ca^{2+} in solution increased the rate of uptake, this effect was stronger with increasing concentrations. Ca^{2+} has also been seen to have this effect on the uptake of other anions (Huffaker and Rains, 1978). The hypothesis behind this effect is that the charge of the Ca^{2+} hides the negative charge of the cell wall reducing the repulsive effect of the negatively charged cell to the anion (Huffaker and Rains, 1978).

NH_4^+ was found to inhibit the uptake of NO_3^- (Huffaker and Rains. 1978). Jackson (1978) showed that NH_4^+ caused the NO_3^- transport system to degenerate in studies on *P. crysogenum.* In another case studied by Jackson (1978) the inhibitory effect was allosteric. These are examples in which the uptake of NO_3^- is decreased, but there are other possibilities depending on plant interactions (Jackson, 1978).

Rate of NH_4^+ uptake is influenced largely by the soil pH, as pH increases so does NH_4^+ uptake. In contrast to this, NO_3^- uptake increases with decreasing pH. Concentration of NH_4^+ in soil solution also influences NH_4^+ uptake (Reisenauer, 1978).

Reisenauer (1978) did trials growing plants in non-soil media. It was found that the uptake of cations in medium with high NO_3^-, but still containing NH_4^+ was lowered. This was attributed to a direct competition between K^+ and NH_4^+. A lower concentration of indiffusable organic anions was responsible for the chelation of K^+ and subsequent decrease in accumulation, as well as lower NO_3^- uptake in presence of NH_4^+ (Haitt, 1978).

Availability of PO_4^{2-} in soils increased at higher pH and with an increase in NH_4^+ which led to a positive result on plant growth (Reisenauer, 1978). Low P availability decreases the nodulation potential and consequently, the N-fixing ability (Barea et al., 1988).

2.2.3.6 Utilisation by plants

N is usually taken up by plants as NH_4^+ or NO_3^- from soil solution. There are some plants that form symbiotic relationships with soil organisms, as discussed previously, to assist the uptake of N from air. Forms of N in soil can change rapidly and leaching occurs easily, consequently so will the availability of N to plants (Blackmer, 2000).

With the absorption of N from the soil, acidification of the root zone occurs; the reason for this seems to be the exchange of NH_4^+ for H^+ from the plant (Chaillou and Lamaze, 2001). There is a relationship between the uptake of one and the release of the other (Van Egmond, 1978). It has also been suggested that acidification could take place due to the discrepancy between the uptake of cations and anions (Huffaker and Rains, 1978). One such example is K^+ uptake in barley, in this case it is possible that H^+ efflux is incorporated into the soil solution (Huffaker and Rains, 1978).

The availability of N to the plant is dependant on the rate of N cycling as well as the content of N in the soil; (Martins-Loução and Lips, 2000) plants can adapt the rates at which available nitrates are absorbed according to their requirements (Touraine and Gojon, 2001). The rate of nitrate uptake increases with time, beginning with a lag phase and increasing towards a linear curve (Touraine and Gojon, 2001). Huffaker and Rains (1978) also showed that pH had an influence on the rate of NO_3^- uptake and that the rate of uptake from buffered solutions in a hydroponic system was lower as time progressed in comparison to unbuffered solutions, where the decrease in pH seemed to have a self perpetuating effect (Huffaker and Rains, 1978).

Inhibitors of protein synthesis decreases the uptake of NO_3^- (Huffaker and Rains, 1978). NO_3^- taken up by plants can be accumulated into root cells for storage, part of which is reduced or translocated into leaves, again a fraction of this will be reduced. The amount of NO_3^- reduced in leaves correlates well to the amount of leaf growth that occurs (Huffaker and Rains, 1978).

2.3. Water quality, N leaching and the effects of alien legume invasion in South Africa

There are different limits for NO_2 and NO_3^- content, either legislated or seen as accepted levels for different water uses. The following are examples of such limits: for recreational purposes, the accepted N concentration is 6.0 to 10 mg/l. The World Health Organisation (WHO), (2004) safe limit for NO_3^- in drinking water is 10 mg/l, whilst the Department of Water Affairs and Forestry, DWAF (1996), has set the "no risk limit" for domestic use as 0 to 0.5 mg/l for both NO_2 and NO_3^-. Also, the accepted concentration of NO_3^- in drinking water was legislated by the U.S. Public Health Service in 1978 as 10 ppm (Lorenz, 1978). For irrigation purposes, class one (high quality) water is given as 0-5mg/l and class two (lower quality water) is given as 5-30mg/l. The target range for the watering of animals is 0-100mg/l for NO_3^- and 0-10 mg/l for NO_2 (DWAF, 1993). High N in water can be a health concern, causing interference with haemoglobin in the blood of small children (Williams, 1999).

Most soils have a low retention capacity for N as NO_3^- because they have low anion exchange capacity, meaning that potentially the addition of water will result in leaching if this ion is present (Pierzynski et al., 2005). The amount of leaching that will take place depends on the volume of water passing through the profile and the amount of nitrate available (Pierzynski et al., 2005). Gunderson et al., (1998) stated that soils with C:N ratio of below 25 on the forest floor have a high risk of nitrate loss by leaching. Nitrification increases at a soil C:N of less than 20 on the sites reviewed by Wilson and Emmett (1998), this statement is supported by Morot-Gaudry and Touraine, (2001). Soils with a low C:N ratio and high %N generally have more nitrification and lower rates of immobilisation leading to a higher risk of nitrate leaching (Wilson and Emmett, 1998).

Leaching of soluble nitrogen is usually a localised problem with sandy, well drained soils and particularly fynbos type soils being the most susceptible (Pierzynski et al., 2005). Other factors increasing the possibility of high nitrate leaching and possible groundwater contamination are shallow water tables, high rainfall or irrigation and high N applications as well as sandy textured soils (Herppich, 2002; Pierzynski, 2005, Neeteson, 1999; Reitz,

1978; Otto et al., 1999). A sufficient anion exchange capacity to decrease N leaching has been recorded in the case of some highly acidic subsoils, but is uncommon and usually does not play a role of great significance (Pierzynski et al., 2005).

Plant litter is a source of N that contributes to the leaching of NO_3 and if decomposition takes place before the rainy season the possibility of leaching is enhanced (Otto et al., 1999; Neeteson, 1999). Vegetation type also affects the potential leaching by differing rates of transpiration; higher leaf area index results in higher transpiration and less water loss by leaching (MacDonald and Jarman, 1984). Fynbos intercepts 5-10% of rainfall, and due to its low biomass transpires little (Versfeld and Van Wilgen, 1986). Plant nutrient uptake also decreases leaching and potential groundwater contamination (Allen et al, 2004). The release of N from litter creates a system where nutrient cycling can be rapid and can potentially be very productive, provided that this N is not lost from the system by leaching (Berg and Staaf, 1981).

Versfeld et al., (1998) placed *Acacia saligna* in the category of medium water use as far as alien invaders in South Africa are concerned. High water use by alien invaders increases economic costs, giving little in return (Versfeld et al., 1998). The access to water resources by many alien invaders is aided by the fact that these are usually located in riparian zones (Versfeld et al., 1998).

Approximately 53% of the Swartland area (232 556ha) is invaded with alien vegetation, of which the condensed invaded area is 13%. Due to these invaders, there is a 39% reduction in mean annual runoff; which totals 114.2 million m^2 of water (Versfeld et al., 1998). *Acacia saligna* is responsible for 136 million m^2 of water loss per year over the Western Cape area and a total of 171.2 million m^2 in South Africa. Of the alien invaders in the Western Cape, *Acacia cyclops* and *Acacia saligna* are found to be the most prolific and they primarily make use of fog and groundwater as water sources (Versfeld et al, 1998). The above-gound biomass with invasion of fynbos vegetation by woody aliens can increase 3-10 times (Versfeld and Van Wilgen, 1986) resulting in decreased water supply due to increased water use and interception by the aliens. In the model designed by Le

Maitre et al, (1996) drastic reductions in available water were predicted on invasion of fynbos vegetation by a few chosen invaders, with a mean reduction value of 350m^3/ha/a. The streamflow reduction that takes place with afforestation, can greatly affect the water supply of the Western Cape, since many of these are catchment areas (Versfeld and Van Wilgen, 1986). Van Wilgen et al., (1992) have estimated that without clearing, woody legumes can invade fynbos catchment areas after four fire cycles.

Bechtold, et al, (2003) found that rainfall events rapidly deplete the soluble N soil store, and that this is the main source of N in surface water. Leaching to subsurface water also occurs in areas further away from streams. They also found that during the dry season, easily mobilised N is stored in the soil and is easily leached once rainfall occurs. Although groundwater contains more soluble N during the high rainfall season, it is the times of slower water events that are more beneficial for productivity, since it is released slower and is more available for absorption (Bechtold et al., 2003). It was found that leaching from a fallow peanut field resulted in contamination of groundwater (Williams, 1999).

2.4. Native and alien vegetation systems affecting N dynamics in the Western Cape

Fynbos systems constitute a very finely balanced system that can easily be disturbed (Olivares et al., 1988). Felker and Bandurski (1979) maintain that the best cultivation crops for such systems are those with low demand on water and nutrients, with high productivity and that do not alter soil conditions. Legumes, planted for grazing, are preferred for areas where soil N is low because of their capacity to fix N and because they can serve as suitable green manure and contribute to soil regeneration (Felker and Bandurski, 1979). *Acacia saligna* has higher nutritional inputs than fynbos and has the ability to change the nutrient dynamics of a fynbos system (Macdonald and Richardson, 1986; Versfeld and Van Wilgen, 1986).

2.4.1 Vegetation changes and soil N

Studies done by Lorenz (1978) showed that, among a selection of vegetable crops grown, the tissues of leaf and stem had the highest NO_3^- accumulation, followed by roots, whilst very small amounts were found in the flowers and fruit. Carter (1978) estimated that if 30 million ha were cultivated with legumes in combination with pasture, there would be a 1.4 million tonne increase in soil N per year (as cited by Cocks, 1988). In Australia, pasture legumes have been shown to add 30-160 kg N/ha to soil each year, and fix a total of 160 kg N/ha (Puckridge and French, 1983). Most legumes planted as grazing used in a trial by Papstylianou (1988), received 60-80% of N by fixation in cases where the nodules were effective.

The introduction of Acacias to the Western Cape was mainly with the idea of stabilising sandy areas (Stirton, 1978), but it has been noted that post-fire, these species can increase the rate of erosion and are not as effective as some indigenous plants in stabilising loose soil (MacDonald and Jarman, 1984). The main effect of invaders on water resources was noted largely as a result of the change in energy balance by interception of light and diffrerence in transpiration rates to a lesser degree. If the leaf area index of invader plants is more than that of the indigenous, leaching of nutrients and drainage will be reduced because of higher transpiration (MacDonald and Jarman, 1984).

As previously mentioned, invasion generally is accompanied by a large increase in above-ground biomass, leading to changes in nutrient cycling, hydrology and fire regimes (Versfeld and Van Wilgen, 1986). *Acacia saligna* is seen to increase litter production and soil nutrient availability (Musil and Midgley, 1990; Musil, 1993). It was noted that species richness of the invaded area is decreased (Macdonald and Richardson, 1986), erosion rates increased, coastal dune movement prevented and diversity of vertebrates reduced (Van Wilgen and Richardson, 1985). Invasion usually increases the available nutrient pool and modifies the nutrient ratios within the pool; the difference in litter accumulation will also alter the soil nutrient status (MacDonald and Jarman, 1984).

2.4.2. Fynbos vegetation system and nitrogen

Cape Floristic Kingdom; the fynbos area of the Mediterranean Western Cape stretching into the bordering summer rainfall area, contain many rare and endangered species and is a vulnerable biome, seriously threatened by alien invaders. Mediterranean environments are dominated by schlerophyllous and evergreen shrubs and trees, strongly leached soils in these climes have been seen to consist mainly of schlerophylls in the over- and understory (Specht and Moll, 1983). The lowland ecosystem and the coastal fynbos regions are still susceptible to further invasion (Holmes and Cowling, 1997; MacDonald and Jarman, 1984) and are very sensitive to human disturbance and changes in nutrient status and water balance (Specht and Moll, 1983). *Acacia saligna* was rated as the most significant alien invader in the lowland fynbos and strandveld (Macdonald and Richardson, 1986). It is widespread and is also a great future threat to the ecosystem and has the potential to negatively affect community processes (MacDonald and Jarman, 1984; Richardson et al., 1992).

Fynbos regions are known for recurring fires, summer drought and nutrient poor soils (Le Maitre and Midgley, 1992). Many invaders have been introduced from areas of similar climates including *Acacia saligna* from Australia (Richardson et al.,1992). Soil nutrient status changes after fires, P and other cations are higher, but N is lower due to loss by volatilisation (Cocks and Stock, 2001). Fires in fynbos regions can increase the N content of soil for a short period (Stock and Lewis 1986), but in the long term, fires will decrease the N availability by volatilisation (Lynds and Baldwin 1998, Herppich et al., 2002).

The leaf N contents of fynbos areas vary; coastal fynbos has less leaf N on average than mountain fynbos. Coastal fynbos has between 54mmol m^{-3} and 212mmol m^{-3}, and mountain fynbos between 64mmol m^{-3} and 303mmol m^{-3} N per leaf. In relation the C content does not vary much between coastal and mountain fynbos (Herppich et al., 2002). In an experiment done by Herppich et al., (2002), leaf N was higher during the rainy season than in the dry summer. According to Maier et al., (1995) this may be due to a temporary sink action of the leaves and relocation of N during the spring growth.

The lower N content in coastal fynbos resultes in an increase in the efficiency of photosynthetic N use (Herppich et al., 2002). Fynbos soils have low inorganic N content due to low mineralisation rates, but the organic N content in relation can be higher by up to 100 times (Stock et al., 1995).

Mitchell et al., (1986) found that at Pella, near Malmesbury in the Western Cape, litterfall occurred during September and February with the largest amounts during periods of strong winds.

2.4.3. Legumes including *Acacia saligna* and nitrogen dynamics

Mycorrhiza are necessary for successful nodulation of legumes where P is deficient. *Acacia saligna* has an extensive root system which has a high nodulation potential and numerous vesicular arbuscular mycorrhizas (Hoffman and Mitchell, 1986). Legumes usually have symbiotic relationships with mycorrhiza in conjuction with *Rhizobium* because they (mycorrhiza) aid in the uptake of poorly mobile nutrients, most importantly, P, which stimulates nodule formation and the *Rhizobium* relationship (Barea et al., 1988).

Mycorrhiza differ in the ability to take up N and stimulate plant growth, but are, unlike *Rhizobium*, are not host specific. For the successful establishment of a relationship with legumes for the purpose of N-fixation, the strain of *Rhizobium* needs to be the suitable species for the relationship (Materon, 1988).

N that has been symbiotically fixed is available to the plant or added to soil solution in the following seasons by release through root exudates, leaching from plant litter or root decomposition (Vance, 1997). Vesicular arbuscular mycorrhizal transport between species and green manure ploughing can also make N available. The degree to which N will be made available depends on the legume (Vance, 1997).

When the available soil N matches the demand of the plant, the relationship between host and bacteria is suppressed and depending on the future N supply, soil *Rhizobium* levels

can become so low that with prolonged duration, the relationship may not be able to re-establish (Postgate, 1978).

Although rare, these symbiotic relationships are not only limited to legumes. Symbiotic relationships with nodulated non-legumes have also been known to occur but this is plant specific (Postgate, 1978). Algal symbiotic relationships can also exist with the result of N-fixation. Associative symbiosis also occurs, mainly within grass species. This is when the organism and the plant species can grow apart, but interaction with relation to N-fixation occurs without infection and nodulation. Casual associations also occur and in such relationships the advantage to one party is almost negligible (Postgate, 1978).

Ismaili and Bentassil (1988) found that in cereal legumes, the effectivity of N fixation decreased with water stress as nodule number decreased during this time. The deep root system of *Acacia saligna* should prevent this from happening (Cowling and Richardson, 1995). The time of harvest can influence the amount of NO_3^- assimilated in the plant because the content of NO_3^- in the plant will change as the season progresses (Lorenz 1978).

Acacia saligna, commonly known as Port Jackson Willow, prevalent in the Cape fynbos region, is a distressing invader and it is long lived, grows as a thicket and is mammal and water dispersed (MacDonald and Jarman, 1984). *Acacia saligna* is found mainly on acidic sandy soils, typical to fynbos, where water is available. In spite of the fact that one of the reasons for introducing the Acacia was to stabilise sandy soils, they have been seen to accelerate erosion along riverbeds (Macdonald and Richardson, 1986). The Acacia causes a decrease in the available groundwater of the invaded area (Versfeld et al., 1998). Litter under Acacia stands are also higher than that of the natural fynbos, by about three times (Milton, 1981). The estimated increase in N and P inputs are about nine times more under the Acacia than under fynbos (Macdonald and Richardson, 1986).There are higher levels of organic matter in the Acacia system than in the fynbos system because of the higher carbon gaining capacities of the Acacia (Stock and Allsopp, 1992). P and N content of Acacia leaves are two to four times more than in fynbos (Milton, 1980).

The use of annual legumes in crop rotation systems is common (Papstylianou, 1988). The reason for this is that legumes in symbiotic relationships with *Rhizobium* have a great capacity to fix N and in so doing increase the N content of the soil (Papstylianou, 1988). N additions in these systems are also increased by the addition of plant litter (Papstylianou, 1988). Afforestation has the ability to increase the NO_x content of a soil (Ndala et al., 2006).

2.4.4. Galls and the impact of *U. tepperianum* on alien vegetation

The introduction of gall forming rust fungus as bio-control of *A. saligna* and other Acacia invaders has caused a decrease in population of these trees by about 80% in 6-7 years (Morris, 1997). Continuing work done by Wood and Morris (2007) showed that with each subsequent year the death rate of infected plants increased at all sites, even in the case of population explosion after fires. With infection there were decreases in phylloid biomass, this decrease diminished with age (Wood and Morris, 2007). Defoliation takes place in infected trees (Morris, 1997). In the majority of sites the seed production was reduced by between 79% and 97% although one site showed an increase of 44%. Fewer pods were produced for each tree size, although few trees lived to be older than seven years, the lower density of trees resulted in larger stem diameters (Morris, 1997; Wood and Morris, 2007).

Several different types of gall- forming organisms compete with the host plant for nutrients, and can assimilate nutrients from the surrounding plant tissue (Stone and Schonrogge, 2003). Spores are produced on the surface of the gall and wind dispersed, galls are produced annually (Old et al., 2000). Fires in these areas stimulate the germination of *A. saligna* seeds; re-infection seems to be rapid following fires (Old et al., 2000; Morris, 1997). Older trees with thick bark can't be infected and the life cycle of a rust is completed on one tree (Old et al., 2000).

2.4.5 Fynbos restoration

Although the invasion of fynbos regions by Acacia is severe, there is still the potential to restore to natural fynbos because there is a representative of each of the major fynbos species in soil as seed (Holmes and Cowling, 1997). The soil nutrient damage that has been done by alien invasion is more of a problem, these soils are now better suited to grasslands than the natural fynbos (Holmes and Cowling, 1997, Yelenik et al., 2004).

According to studies done by Blanchard and Holmes, (2008) restoration of natural fynbos vegetation after removal of the alien vegetation (by fell and remove in this case) is realistic. Recovery is less when the vegetation is not removed or is burned (Blanchard and Holmes, 2008).

An added difficulty, as previously mentioned, with regards to restoration is that the shift in N cycling from low to high due to the introduction of the alien legume facilitates the growth of weedy grasses (Yelenik et al., 2004). Yelenik et al., (2004) and Holmes (2008) suggest measures be taken to decrease the soil available N in an attempt to increase potential restoration to natural fynbos. This is supported by trials done by Lamb and Klaussner, (1988) where it was found that on two chosen fynbos species N resulted in a negative vegetative growth response. Yelenik et al., (2004) also stated that there were higher growth rates of grasses after the removal of Acacia.

Higher levels of NH_4 and NO_3 in soil were found when Acacia invaded areas were compared to the natural fynbos (Yelenik et al., 2004). The higher N content in the soil was not only due to the N-fixing properties of the invader, but also the large amounts of N-rich litter (MacDonald and Jarman, 1984; Yelenik et al., 2004).

The long- term fynbos seed bank is smaller for lowland fynbos than mountain fynbos (Holmes, 2002). Lowland fynbos will recover slower than mountainous fynbos, and some of the long term seeders possibly not at all (Holmes, 2002). Holmes (2008) has suggested that more than just weed control is necessary for the restoration of fynbos, but that it may

be necessary to sow seeds or replace plants. The necessary actions for fynbos restoration are listed by Holmes (2008) as:

1. Clearance and continual control of competing vegetation
2. Control of smaller weeds
3. Decrease in the soil N content
4. Control of small disruptive mammals
5. Protection of the fynbos soil stored seed bank

All of the above are necessary for successful restoration. Continual control of *Acacia saligna* is important because it has been shown to have high seed banks (Holmes, 2002) and seeds are hardy and long-lived (Holmes et al., 1987).

Fynbos soil seed banks decrease with invasion and more so with increased length of invasion (Holmes, 2002). Short lived fynbos species seem to have better soil seed stores than long lived fynbos species (Holmes, 2002). This difference in soil seed stores is more pronounced in the lowland fynbos than mountainous fynbos (Holmes, 2002).

As previously mentioned, the invasion of *Acacia saligna* in the fynbos environment caused an increase in N input into the ecosystem by higher N-rich litter fall than that of the natural fynbos vegetation (Witkowski, 1991a). Yelenik et al., (2007) also found that Acacia caused higher N inputs from the deposition of N-rich litter than not only the fynbos, but also other grass type invaders. The Acacia also increases the total N pool, this is not the case with the grasses studied by Yelenik et al., (2007), in spite of their higher litter deposition per annum. This is possibly due to slower decomposition by the Acacia, also the N in Acacia litter seems to be fixed by "low quality carbon" (Yelenik et al., 2007). Acacia litter does not immobilise N (Witkowski, 1991a).

2.4.6 Restoration ecology

Seedlings of *Acacia saligna* grow faster than fynbos and this in conjuction with the ability to grow taller (more than three meters) assists the success of invasion (Holmes and Cowling, 1997). Also contributing to the invasion of aliens is that they arrived in the area

without any natural predators or pests; aiding their better seed bank and germination. Among the negative effects of *Acacia saligna* and other invaders is the reduced flow of water in fynbos biome rivers (Holmes and Cowling, 1997). Genetic stock of natural fynbos and medicinal plants is decreased and erosion increases after fires (Holmes and Cowling, 1997).

Fire frequencies tend to increase after invasion and the ability of alien invaders to adapt and regrow after fires, and with changes to new fire cycles, contribute to their success in invading. MacDonald and Jarman, (1984) state that spring and summer fires are unfavourable for invasion, this is because of the lower seed survival of *Acacia saligna* during such dry summer conditions. Usually these seeds can last for decades (Richardson et al., 1992). In spite of this, the use of fire as control would be destructive to the fynbos diversity (MacDonald and Jarman, 1984).

A. saligna out-competes the natural fynbos and becomes the primary vegetation, more so after each fire which are common to these areas; by sprouting with more vigour than the natural fynbos even though most species recruit after fire (Holmes and Cowling, 1997). Invasion by *A. saligna* decreased the biodiversity of the area, almost eliminating and replacing fynbos entirely, in quality and quantity as canopy cover. It also decreases the fynbos seed bank, although the loss found in the seed bank was not so low that reclamation after long periods of invasion would be rendered impossible (Holmes and Cowling, 1997).

Biological- and chemical control as well as felling and burning can be used in combination to control alien invaders (Van Wilgen et al., 1992). Le Maitre et al., (1996) have estimated the costs of control, assuming fires occur regularly every 15 years, would be R33/ha per annum. The possible water saving should be weighed against this.

The recovery of fynbos after the removal of the invader plant varies with the degree to which invasion occurred, but the number of fynbos species is usually reduced (MacDonald and Jarman, 1984). Soil parent material may determine the ability of

different fynbos species to recover after clearing of aliens (MacDonald and Jarman, 1984). Fynbos species recovery was higher in areas where the duration of invasion was shorter (Holmes and Cowling, 1997). Resprouting was negligible in long invaded stands but was responsible for 50% of canopy cover in recently invaded stands (Holmes and Cowling, 1997)

According to Witkowski, (1991a) the increased N due to alien invasion may not be long term after removal of the alien vegetation due to the high sand content of these fynbos soils (Stock and Lewis, 1986). The total litter production under mixed fynbos is 200mg/m^2 per annum, as observed by E.J Moll on the fynbos in Bains Kloof (Read and Mitchell, 1983) and 84 and 71.5 g/m2 for two consecutive years at Pella as studied by Mitchell et al.,1986. These values will vary with the time elapsed since last fire event (Versfeld and Van Wilgen, 1986). Decomposition rates will depend on the quality of the litter and environmental conditions. Schlerophyllous plants produce poor nutrient litter (Read and Mitchell, 1983). In the case of schlerophylls the amount of nutrients that are replaced into the system are minimal, some nutrients, particularly N and P, are re-assimilated into the plant prior to abscission (Mitchell et al., 1986). Decomposition of organic litter is slow and as the age of the stand increases, so will the litter layer, Mitchell et al., (1986) conclude that the major nutrient release under fynbos vegetation is not by litter decomposition, but from fires.

2.4.7 Nutrition and plant responses of fynbos and Acacia

Protea repens had increased growth response to increased nutrient contents (different levels of P and N) in experiments done by Witkowski (1991b), but when faced with the competition of *Acacia saligna* under the same elevated nutrient competition the growth response was not competitive. Under lower nutrient content with competition *P.repens* did better than the Acacia and there is the possibility of out-competing it (Witkowski, 1991b). The levels of comparative uptake of N and P by alien seedlings were higher than the fynbos seedlings by an estimated 100%, but more efficiently at higher nutrient levels (Witkowski, 1991b).

Similar studies done by Lamb and Klaussner (1988) found that the Protea had a negative vegetative growth response to nutrient additions especially N, and N and P in combination. The Erica studied had a positive response to increased P levels, but decreased to increased N (Lamb and Klaussner, 1988).

Acacia increases available and total soil N (Yelenik et al, 2007). One of the problems with the rehabilitation of fynbos is many of the invaders specific to fynbos areas thrive under higher N contents caused by Acacia invasion (Yelenik et al., 2007; Witkowski, 1991a; Yelenik et al, 2004).

Chapter 3: Changes in soil properties due to alien legume (*Acacia saligna*) invasion and clearing

3.1 Introduction

Soil nutrients status changes occur with the invasion of an alien species (Stock and Allsop, 1992). It has been found that the invasion of Acacia in fynbos areas causes a shift from low to high N cycling (Yelenik et al., 2004). The resulting nutrient status is more suited to grasslands than to fynbos and could negatively affect the natural ecosystem (Holmes and Cowling, 1997, Yelenik et al., 2004). In studies done by Yelenik (2007) the N-rich litter and greater quantities of litter added by the Acacia caused higher N inputs into the system than that of the natural fynbos. Previous studies have been done on the removal of alien vegetation and the effects on nutrient status. The focus on NO_X in these studies is insufficient to allow any conclusions to be drawn. The objective of this section of the study is to focus on the basic nutrient changes due to alien invasion by *Acacia saligna*, the subsequent removal as well as the effects of rainfall on these properties. Studies on the effect of alien invasion on soil nutrient status have been done in the past, but at this point in time little is known about how the removal of such invaders change the soil and water nutrient status, particularly with regards to N.

3.2 Materials and methods

3.2.1 Site description

These studies were done at Riverlands Nature Reserve; the reserve is near Malmesbury, off the N7 about 60km out of Cape Town. A map with co-ordinates outlining the sites selected can be found in appendix A. The reserve was well suited to the study due to the presence of a long invaded and homogenous Acacia stand that was already scheduled for clearing by Working for Water (WFW). The sandy soils and underground aquifer provided the appropriate site for sample selection so that soil and underground water

(results reported in chapter four) could be studied according to the objectives. Riverlands Nature Reserve itself has mostly natural fynbos vegetation but much of the surrounding area is densely populated by the alien invader *Acacia saligna.* Three vegetation sites were used for this study. The natural fynbos vegetation was studied as a control, an alien invaded, Acacia site on the neighbouring farm, and a site cleared of the alien invader during the study.

The site selected for study relating to fynbos vegetation was chosen because it is rich in diversity of species and represents natural fynbos vegetation. An advantage is that it is 11-15 years old where much of the surrounding fynbos is new since fires swept through in 2004 and 2005. The most common shrubs found on the site selected are of Proteaceae, Ericaceae and Rutaceae. *Euclea racemosa* and *Diospyros glabra* are also prevalent. Although the site is on a slope with a difference in elevation of under 0.5m, there is a difference in the vegetative community, roughly split into restio in the low-lying areas and protea in the high-lying areas. This is another reason why this site is a good representative of the general naturally growing fynbos community and specific studies of N within each individual species is not necessary to the findings of this study. This would, however, be interesting grounds for further study.

The Acacia site was separated from the fynbos site by a distance of 50 metres. Acacia trees were found in clusters with weedy grass and some herbaceous species in open patches, but these were sparse. Average canopy cover by the Acacia was 45%. Most of the ground was covered by Acacia litter. Trees varied in age varying from saplings to dead trees, but no seedlings were found. The land was used for cattle grazing until fairly recently before the study and this could explain the lack of seedlings. Trees were in the range of 4 to 7 meters tall and most branches were infected with the parasitic gall *Uromycladium tepperianum* which was introduced as a biological control.

Clearing was done on an Acacia site identical to the one described above. Acacia trees were cut down and left on the ground to imitate the practice most commonly used by farmers.

The climate is Mediterranean with an average rainfall of approximately 440mm per year, most of this occurring between May and August. Although during the rainy season water is seen to accumulate on the soil surface of the lower lying areas. For the most part evaporation is more than rainfall. Summers are warm with a mean daily temperature of 27.9°C in February, compared to 7°C in July.

3.2.2 Experimental design

Soil samples were taken every fortnight from May to November 2007 at approximately the same time of day (afternoons between 2 and 5) and once more in early December 2007. Samples were taken using a Jarrett soil auger and three replicate samples were taken per site, per sampling date at three soil depths; 0-5cm, 35-45cm and 75-85cm. The replicates were not analysed individually, but rather averaged physically by mixing. As a result the analysed sample represented three sampling points for each depth increment of every test area. To determine the validity of physical averaging a separate experiment was undertaken. All three replicates were analysed separately on one of the sampling sessions and the results were averaged statistically for comparison with physically averaged samples. These composite samples were seen to correspond well to the statistically averaged samples (Results are shown in figure 4-2 and discussed in section 4.3.2).

Samples were collected along the slope, in an approximately north to southerly direction for the fynbos site and South to Northerly direction for the Acacia and cleared sites. Examples of each site of vegetation were not replicated because the soil samples within the sites were replicated. This resulted in a pseudoreplicate design, although emphasis was placed on the differences between sites, soil samples within each site were replicated. Unfortunately this has prevented the use of extensive statistical testing.

Soil samples were stored wet and under cool conditions before being air dried, sieved and analysed. Samples were incubated so that N concentrations may stabilise. Sieving and

improved air circulation may increase mineralisation in stored soil samples (Nordmeyer and Richter, 1985), in this way the maximum mineralised N can be determined and this is of relevance to determine the potential groundwater pollution. In this way the mineralised N may be overestimated, but if the mineralised N had been underestimated, that would be a problem for this study, resulting in lower estimated levels of N for leaching to groundwater. The difference between the maximum mineralised N and mineralised N before storage is expected to be insignificant because of the total N that is so much higher.

3.2.3 Soil chemical analyses

Soils were air dried and sieved through a 2mm sieve. pH was measured in distilled water and in 1M KCl from a 1:2.5 solution and EC was measured in a 1:5 soil water solution. pH and EC were determined for all samples, replicates and dates separately, according to the methods outlined in Mc Lean, (1982) and Rhoades, (1982). Total carbon and nitrogen content as well as the levels of basic cations, Ca, Mg, Na and K were determined for three selected dates. Due to financial constraints and the level of relevance for the study they were not analysed for each sampling date. The selected sampling dates were; pre clearing (8 May 2007), soon after clearing (24 June 2007) and at the end of the sampling season (9 December 2007). Total carbon and nitrogen were determined by combustion (gas chromatography) as in the method outlined by Bremner and Blackmer, (1982). The sample was milled and weighed into a metal cup in preparation for analysis by gas chromatography. Cations were measured from a 1:4 soil NH_4OAc extract (as in the method described by Rowell (1995)), and analysed by atomic absorption spectroscopy (Thomas, 1982; Knudsen et al., 1982).

Once it was checked that composite samples were true representatives of the average of the replicates, the samples were used for the analysis of anions. Composites of the three replicates were made from a 1:5 soil water extract. This was done for each sampling date and soil depth, and then used for anion determination by ion chromatography (Rhoades, 1982). Raw data is tabulated in Appendix C.

3.3 Results

3.3.1 Soil description

Soils of the study sites are coarse sandy soils with a weakly developed structure and good drainage. Their origins of these soils are marine and Aeolian. Soils were described according to the SA classification system. Under the natural fynbos vegetation the soils are weakly developed podzols, mainly of the LaMotte soil form. They have Podzol B horizons and some have E horizons, this varying over the site. Lower on the slope gleyed properties can be seen. Under the alien vegetation the soils have orthic A horizons with a clearly bleached E horizons as well as a weakly structured B horizon (neocutanic or yellow brown apedal B). These fall into the Constantia and Vilafontes soil forms according to the South African soil classification system (Soil Classification Working Group, 1991). The soil classification is summarised in Appendix B.

3.3.2 pH and EC in sandy soils

There was a decrease in pH (as averaged over the sampling period) with depth distribution on the cleared and Acacia sites as seen in Figure 3- 1, but under fynbos vegetation pH increased with depth. Over the season there was little change in pH, and the pH values are only slightly acidic.

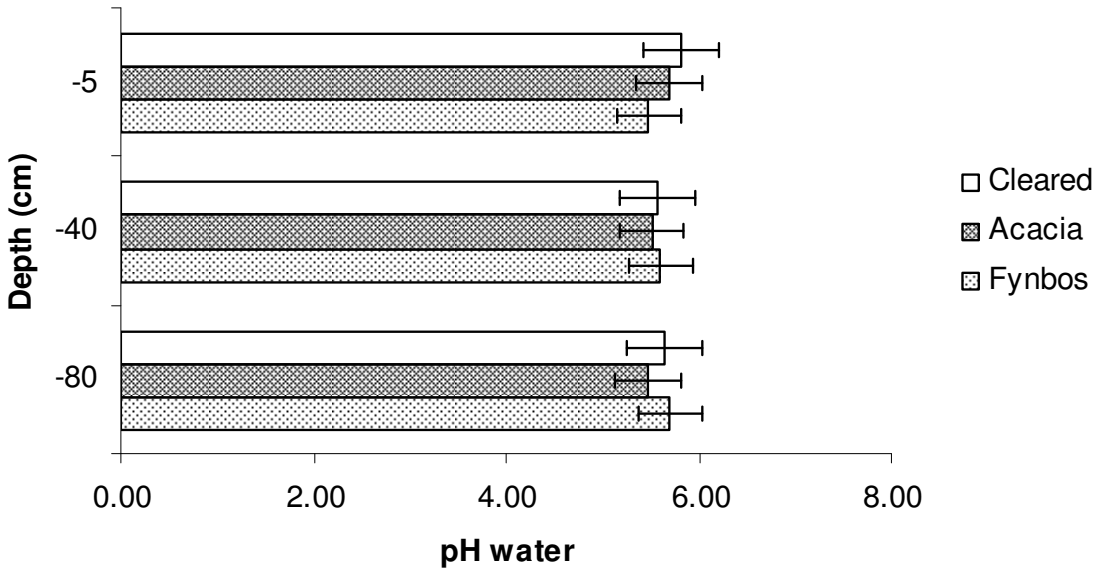


Figure 3- 1: The pH averaged over the sampling period for the three soil depths at the three different study sites, error bars denote standard deviation.

The depth distribution of the EC values averaged over the sampling season of the soils of the Riverlands Nature Reserve is plotted in Figure 3-2. These soils have EC values of less than 30µS/cm. The decrease in EC with depth is most clearly seen under Acacia vegetation. On the cleared site there was an initial decrease and subsequent increase in EC with depth. Under Fynbos vegetation there is almost no change with depth, but with a trend similar to that of the cleared site. EC correlates well with the sum of cations which is represented in Figure 3-3 in the same manner as EC. The relationship between cations and EC has an r^2 of 0.603.

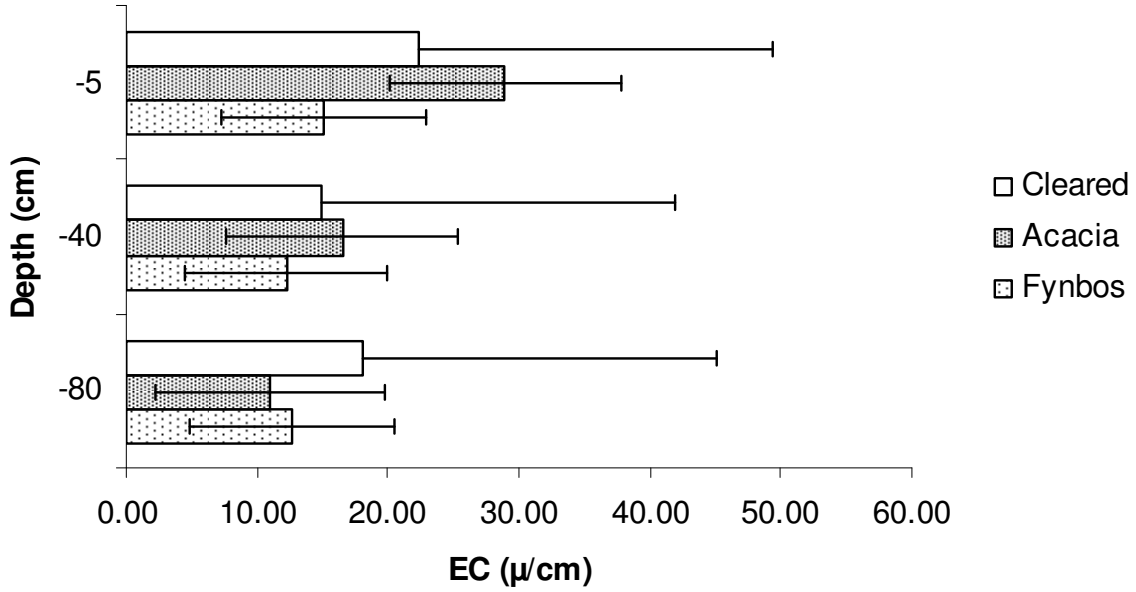


Figure 3- 2: The EC (µS/cm) averaged over the sampling period for the three soil depths on the three different study sites, error bars denote standard deviation.

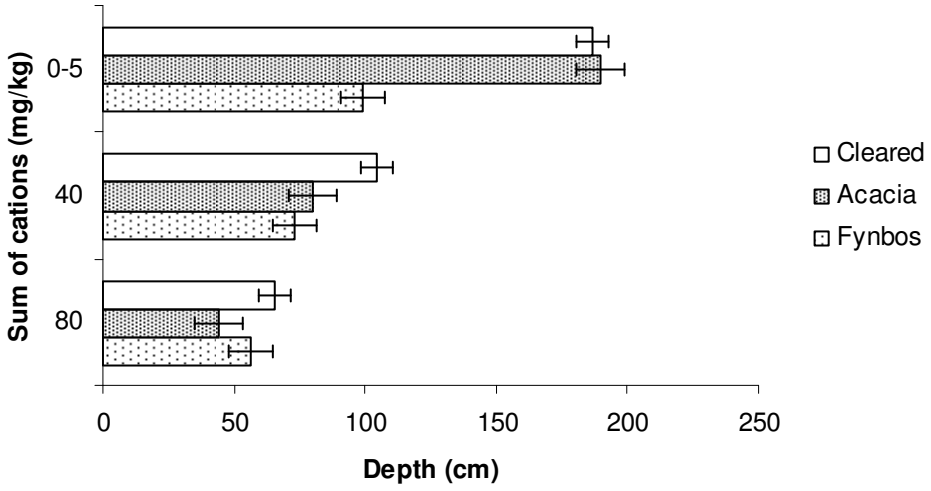


Figure 3- 3: The averaged sum of cations (mg/kg) over the sampling period (2007) for the three soil depths on the three different study sites, standard deviation has been denoted by the error bars.

3.3.3 Cations

Cations are easily leached and with leaching the soil pH usually decreases. The sum of cations is plotted against the pH in water for the Riverlands soils in Figure 3-4 and, although there is not a wide range, there is a slight increase in cations at higher pH's. Trendlines have been drawn to help predict the trend to increase or decrease over the season. These are not regression lines and as such, no R^2 value or equations have been included.

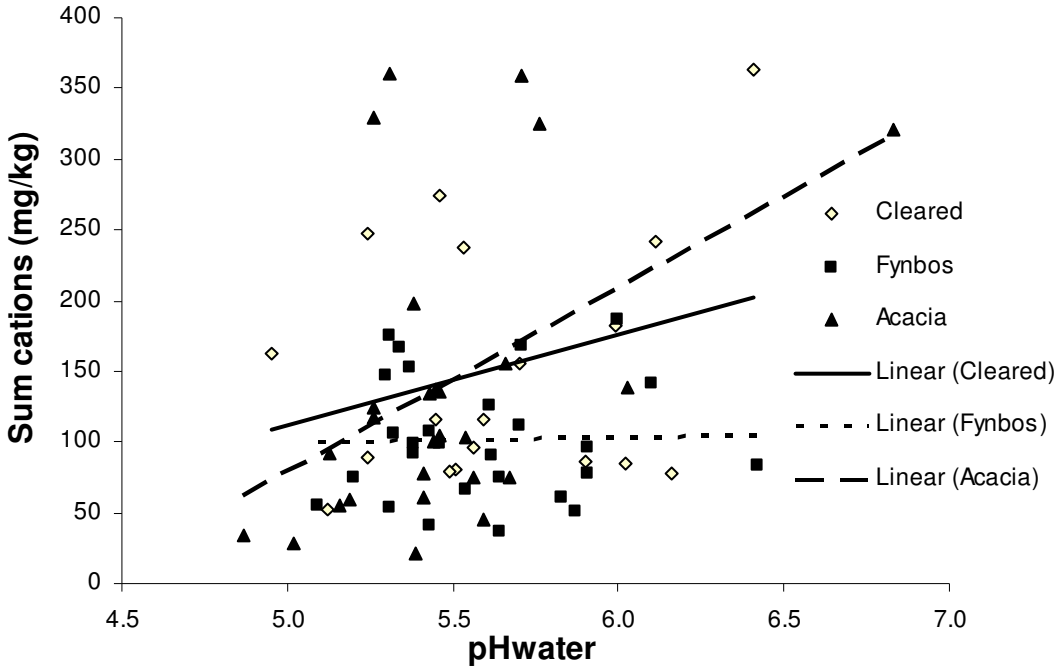


Figure 3- 4: The sum of cations (mg/kg) for the three study sites and their relation to pH.

Clay contents of the three soils were determined from soils extracted during the drilling of well points; Silt and clay distribution is plotted in Figure 3- 5. The difference in clay content is small, less than 1% and each soil has a low clay content being 1.9% under fynbos vegetation. The trends are similar for all sites with the differences owing to depositional lamellae. There is an elluvial layer higher up, followed by an illuvial layer. The Acacia site was higher in altitude than the other two sites and shows this in the

higher silt + clay at 35-45cm where the other sites experience an increase at about 75-85cm.

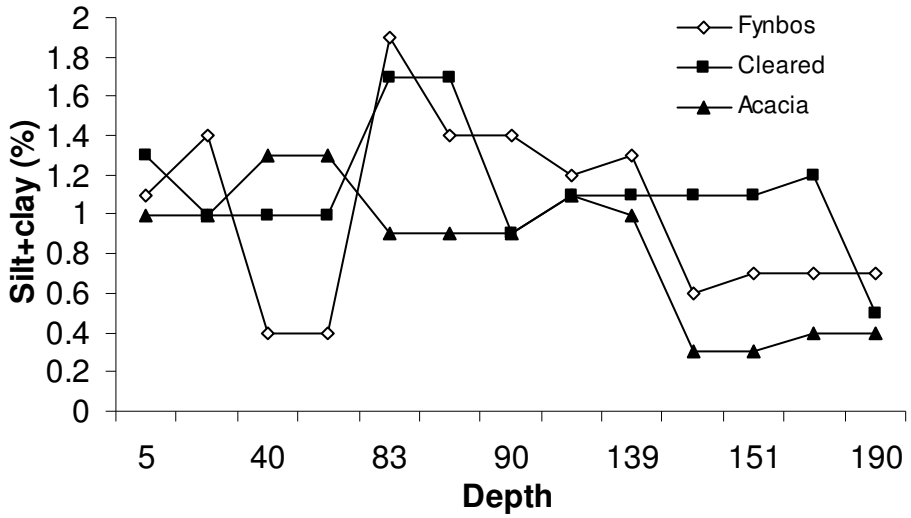


Figure 3- 5: Silt+clay distribution with depth on the three sampling sites

Figure 3-6 a, b and c show the change in the sum of cations at soil depths 0-5, 35-45 and 75-85cm at the three selected dates during the sampling period.

At 0-5cm there is a decrease in cation content over the season, pH also decreases in a similar manner over the sampling period. The fynbos values are far lower than that of the other areas, with a slight increase in cation content nearing the end of the season for all soil depths. Overall values are higher at 0-5cm compared to the other soil depths.

At 35-45cm there is an initial decrease in cation content and a subsequent increase, this is the case for all sites, although the increase as well as the total values on the cleared site are larger than for the other sites.

After an initial drop in the cation content on the fynbos and Acacia sites at a depth of 75-85cm, there is a slight increase at the end of the season. The trend on the cleared site is

vastly different with little change, but a decreasing trend in cations over the season. The total cations under the cleared site are more than those of the other sites except for the initial higher values under fynbos.

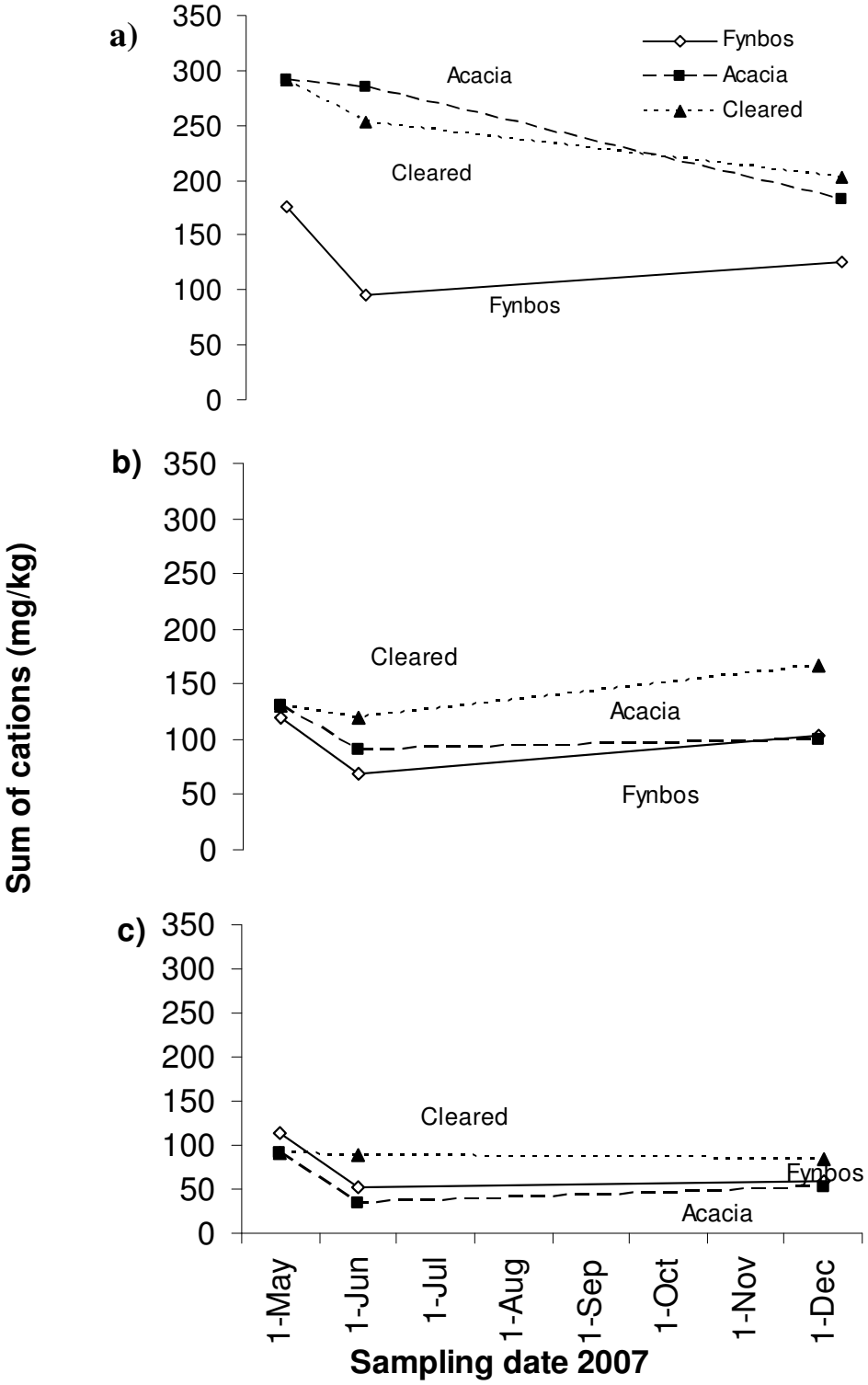


Figure 3- 6: Change in cations (mg/kg) at three selected dates over the sampling period (2007) at soil depths a) 0-5cm, b) 35-45cm and c) 75-85cm.

3.3.4 Total carbon and nitrogen

A strong linear correlation between carbon and nitrogen is expected, and can be seen in Figure 3-7. The C:N ratio of the Riverlands soil is on average 6.5, this is lower than in most soils. The accuracy of the EuoEA3000 series used for the determination of total carbon is 0.1% by weight and 0.02% by weight for nitrogen (CAF, 2009), this is high and should eliminate doubts concerning these low C:N values. Split for each site, the average C:N ratio is 5.2 for the fynbos site, 5.9 for the Acacia site and 7.3 for the cleared site. Again the lines shown in figure 3-7 are trendlines and not regression lines.

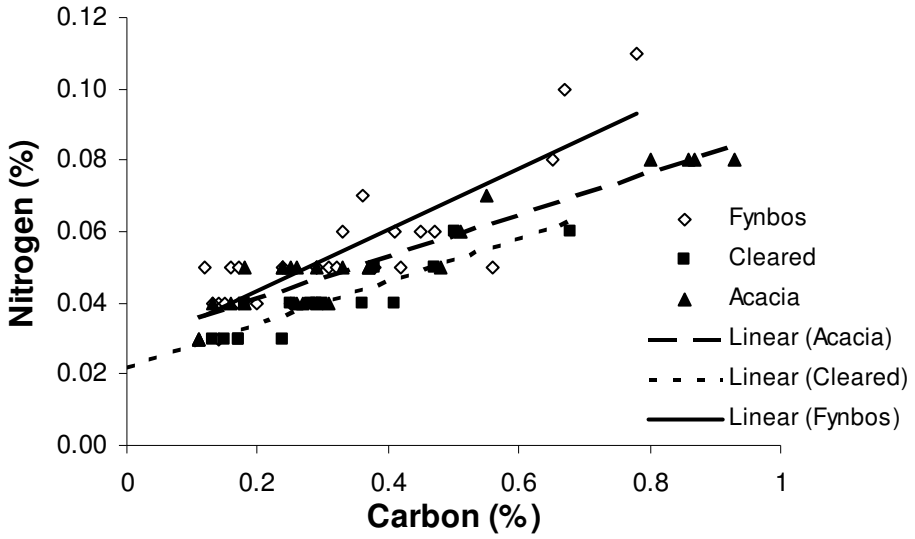


Figure 3- 7: Linear correlation of carbon (%) and nitrogen (%) for the three different study sites.

3.3.5 Mineralised N

The C:N ratios in these soils are narrow. Soils of the Riverlands Nature Reserve that have been previously analysed have had wider C:N ratios. In previous studies lower total N in surface soil by less than 10 times and lower mineral N by 100 times have been reported (Stock and Lewis, 1986). The C:N ratios found in this study are not unrealistically narrow, C:N ratios of soils in the Bengal basin have been reported to have C:N ratios of between 2 and 11 (Datta et al., 1999). Datta et al (1999) ascribe the narrow C:N ratio to

the large amounts of inorganic N. In an experiment done by Yan, et al, (2008), NH_4^+ in soils with narrow C:N ratio, remained in soluble and easily accessible forms, where NH_4^+ in soils with a wide C:N ratio was immobilised fairly quickly. The soluble N has been subtracted from the total N for this study to determine whether the C:N ratio could be narrow because of a high soluble N content. The percentage of mineralised N of the total, in the soils studied at Riverlands has been determined and is presented in The % of mineralised N varied from 0.1 to 6.2.

Table 3- 1: % of nitrogen in mineralised form

Sample details			Total N	C:N	N, mg/kg in			Mineral N	
Date	Site	Depth	(%)		NO_2	NO_3	∑	N (%)	% of Total N
8 May	Fynbos	5	0.07	8.25	5.16	0.00	5.16	0.00052	0.8
8 May	Fynbos	40	0.06	5.00	3.52	0.00	3.52	0.00035	0.6
8 May	Fynbos	80	0.04	3.31	0.00	2.22	2.22	0.00022	0.5
8 May	Acacia	5	0.07	9.33	0.43	21.62	22.05	0.00220	3.1
8 May	Acacia	40	0.09	3.07	0.00	6.50	6.50	0.00065	0.7
8 May	Acacia	80	0.04	4.42	0.00	2.16	2.16	0.00022	0.5
8 May	Cleared	5	0.07	9.33	0.43	21.62	22.05	0.00220	3.1
8 May	Cleared	40	0.09	3.07	0.00	6.50	6.50	0.00065	0.7
8 May	Cleared	80	0.04	4.42	0.00	2.16	2.16	0.00022	0.5
24 Jun	Fynbos	5	0.05	6.53	0.21	5.36	5.58	0.00056	1.1
24 Jun	Fynbos	40	0.04	5.67	0.26	5.43	5.69	0.00057	1.4
24 Jun	Fynbos	80	0.04	4.00	0.00	1.55	1.55	0.00015	0.4
24 Jun	Acacia	5	0.08	10.17	0.37	17.64	18.00	0.00180	2.3
24 Jun	Acacia	40	0.04	6.83	0.00	3.26	3.26	0.00033	0.8
24 Jun	Acacia	80	0.03	4.00	0.00	1.45	1.45	0.00014	0.4
24 Jun	Cleared	5	0.05	9.94	0.00	33.28	33.28	0.00333	6.2
24 Jun	Cleared	40	0.04	7.09	0.00	4.28	4.28	0.00043	1.2
24 Jun	Cleared	80	0.03	5.22	0.00	3.27	3.27	0.00033	1.1
9 Dec	Fynbos	5	0.08	4.48	0.00	1.22	1.22	0.00012	0.2
9 Dec	Fynbos	40	0.07	7.25	0.00	1.23	1.23	0.00012	0.2
9 Dec	Fynbos	80	0.07	6.50	0.00	0.97	0.97	0.00010	0.1
9 Dec	Acacia	5	0.05	4.36	0.15	2.47	2.62	0.00026	0.6
9 Dec	Acacia	40	0.05	7.93	0.06	1.95	2.01	0.00020	0.4
9 Dec	Acacia	80	0.05	7.07	0.00	1.26	1.26	0.00013	0.3
9 Dec	Cleared	5	0.04	5.55	0.09	2.62	2.71	0.00027	0.7
9 Dec	Cleared	40	0.05	8.50	0.00	2.17	2.17	0.00022	0.4
9 Dec	Cleared	80	0.04	8.25	0.00	1.76	1.76	0.00018	0.4

3.3.6 Temporal effects on anions, EC and NO_X

During the season of study there is a response in the sum of anions in the soil at a depth of 0-5cm for each site in relation to rain as plotted in Figure 3-8 a, b and c. A moving

average trendline was added since the difference in x-axis values between the rainfall dates and the dates of sampling would not allow for the plotting of a continuous line joining the data points directly. The same was done for Figure 3-9. The EC and anion trends are similar for all sites. At the beginning of August there was a rainfall event of 78mm, as the rainfall decreased after this, so did the anion content. The fynbos site has lower EC and anion values. Under the Acacia the initial dry period is coupled to an increase in EC and anion content. This can also be seen on the cleared site, but to a lesser degree and with a high peak at clearing. The same trend can be seen with the sum of NO_X and rainfall in Figure 3-9 a, b and c. The results are reported as NOx and not with NO_2 and NO_3 separately, because of the very low values of NO_2 present, and absence in many cases (See appendix C- raw data).

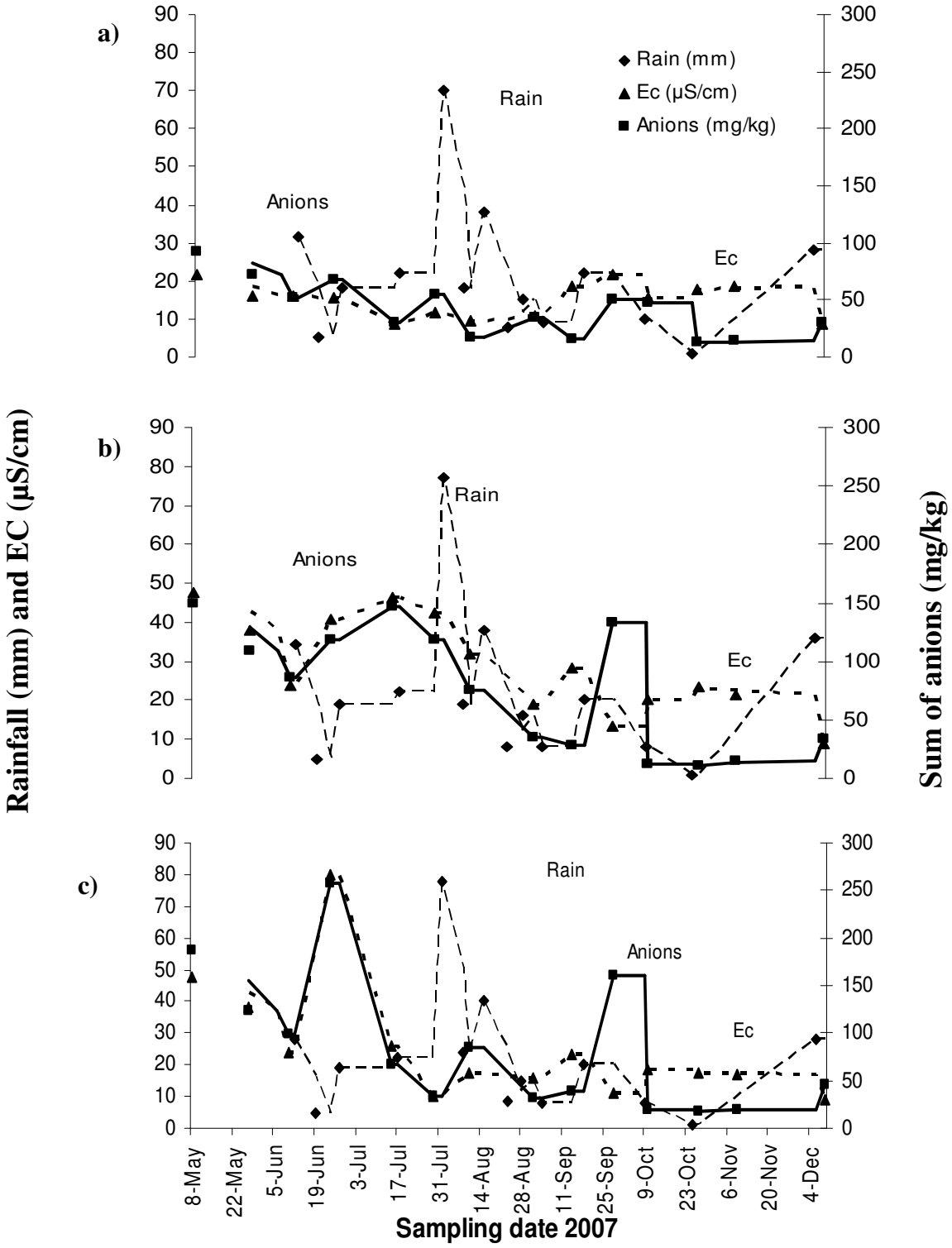


Figure 3- 8: Change in sum of anions (mg/kg) over the sampling period (2007) related to rainfall (mm) and EC (µS/cm) at a depth of 0-5cm on the a) Fynbos, b) Acacia and c) Cleared site

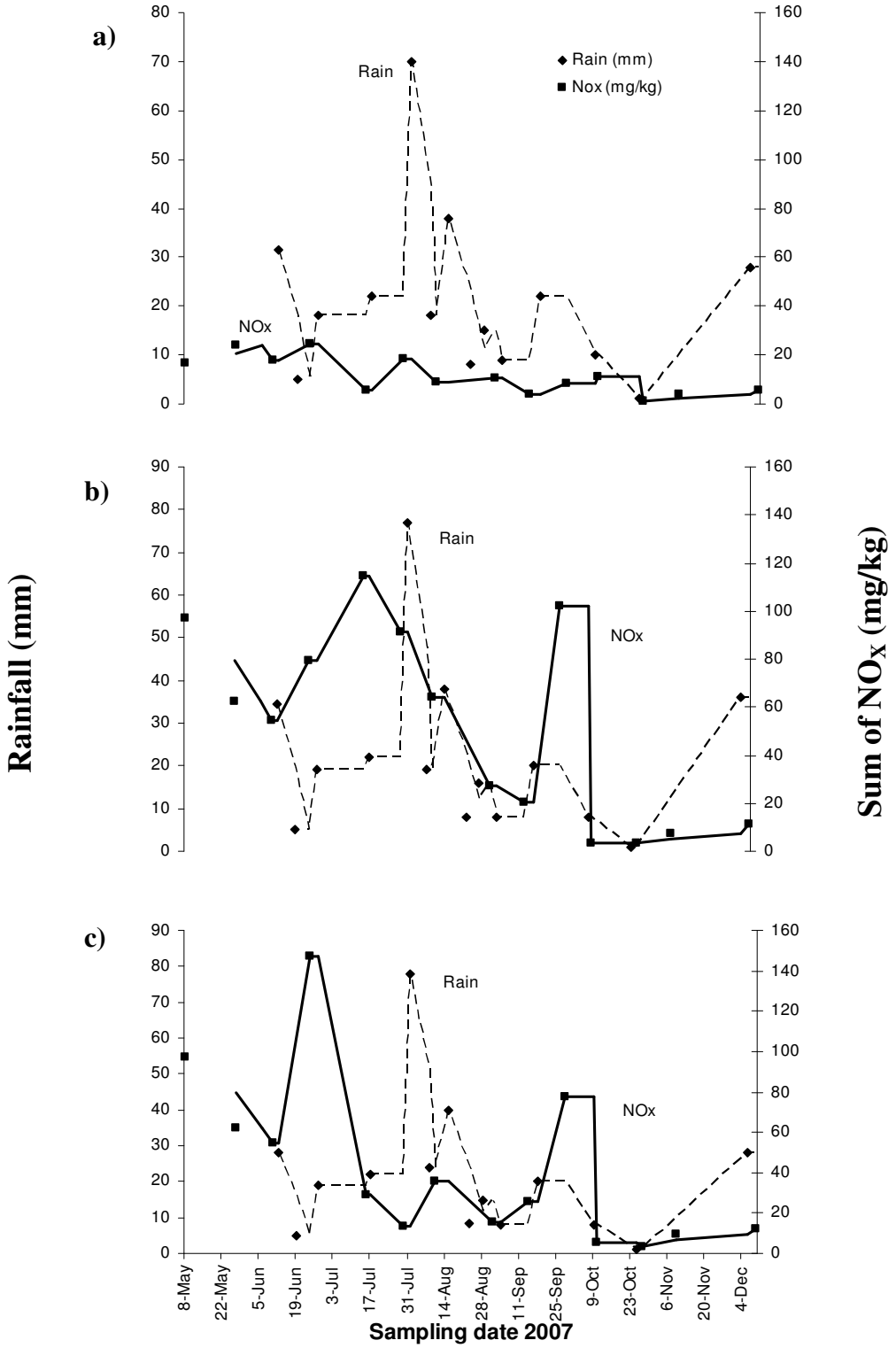


Figure 3- 9: Change in sum of NO_X, (mg/kg) (NO_3^- + NO_2^-) over the sampling period (2007) related to rainfall (mm) at a depth of 0-5cm for the sites a) Fynbos, b) Acacia c) Cleared site

3.4 Discussion

3.4.1 Site differences

The pH decreased with depth on the cleared and Acacia sites, showing progressive leaching with depth. This is to be expected on the cleared site since sandy soils are susceptible to leaching and there was no longer vegetation to decrease leaching. pH under Fynbos vegetation increased with depth and did not show progressive leaching. The pH's of soils on all three sites studied are lower than 6, this was found to be characteristic of fynbos soils by Lamb and Klaussner (1988).

An EC of about 4mS/cm (or 40µS/cm) is the lower limit of salinity (McBride, 1994). The decrease in EC with depth, most clearly seen under Acacia, shows a decrease in the amount of soluble compounds with depth. Under fynbos vegetation there is almost no change with depth. On the cleared site initial leaching occurs with an accumulation of nutrients at 75-85cm depth, this occurrence is possibly because there is no vegetation to use the nutrients. There is good correlation between the sum of cation and the EC change with depth, as is expected.

Usually the relationship between exchangeable cations and pH would be linear as in the cases studied by Hochman et al., (1992), this is in line with the findings in this study. There is little change in sum of cations with pH change under fynbos vegetation, this indicates that the fynbos soil is not well buffered. The Acacia soils were the most buffered, this is probably due to the higher organic matter found on the site. Another possibility is higher clay content, but the difference in clay content between all the sites is only about 1%.

Although the vegetation left on the surface after clearing is a higher organic input than under natural situations, there was no additional input of plant litter after vegetation clearing occurred and only the litter that remained on the surface could have contributed to the higher buffering of cation content.

Higher cation values at 0-5cm were found for all sites and are expected since this is where organic matter breakdown occurs. The decrease of cations at 35-45 and 75-85cm with a subsequent increase over time shows leaching. We can conclude that there is bioaccumulation at 0-5cm and leaching lower down in the profile for all sites. Fynbos values are lower than the other two sites, as is characteristic of fynbos soils, and the cleared site has the most cations due to no plant demand.

The median for the C:N ratio of most soils are expected to be more than 15 and podzols usually have a median of more than 20 according to Snowdon et al (2005), which makes the median C:N ratio of the Riverlands soil (6.2) low. Such a low ratio indicates well decomposed organic material and N-rich litter input as found is true for *Acacia saligna* (Yelenik et al, 2004). The Acacia site had a higher C:N ratio than the fynbos site, this is because fynbos litter is lower in N and fynbos have lower litter inputs. The cleared site had a higher C:N ratio, most likely due to the large amounts of organic litter left on the surface after clearing as well as the removal of most nutritional demands.

The equipment used has an accuracy of 0.1% by weight (CAF, 2009) the values reported I table 3.1 are lower than this, this could explain the unusually low C:N reported. The N values found are lower than the 0.5-3% found at Pella near Riverlands by Stock and Lewis (1986).

The change in anions and EC under fynbos vegetation is low and consistent with the other properties of these soils, there seems to be very little change in these two parameters with rainfall events. The Acacia site has an initial increase in sum of anions and EC with no rainfall, because as the soil dries out there is an accumulation of anions and a coupled increase in the EC. Once rainfall occurs the anions are leached from the topsoil and EC of the top 0-5cm layer decreases with the anion content. This relationship is seen on the cleared site as well. The difference between the trends on the two sites is a peak in anions and EC just after clearing and preceding rainfall the rainfall event in July.

Rainfall stimulates the decomposition of organic matter and can also add NO_X by leaching from the plant canopy. Waterlogging can also decrease plant growth and nutrient uptake. Again the response of NO_X to rainfall on the fynbos site is low and the trend for NO_X is similar to that of anions for all sites. On the cleared site there is a response of NO_X to rainfall, indicating that even though the vegetation has been removed there is NO_X in the system and it can remain for a period after clearing. In an experiment done by Agrella et al., (1999) it was determined that rainfall patterns had the most influence on mineralisation and nitrification rates, the reason for this was because of the stimulation of microbial activity

3.4.2 Changes in pH, EC and the sum of cations with depth.

For the fynbos site there was little change in pH or EC with depth. The pH increased slightly with depth but there was little variation in exchangeable cations coupled to it. Although nutrient levels are lower than on the other two sites, there were significantly more nutrients in the top 0-5cm soil than deeper down.

For the Acacia site, pH, EC, and cations decreased with depth, this site showed progressive leaching of cations. The decrease with depth could also be due to uptake by plant roots. The trend of cation change over time was the same for all soil depths, a decrease over the season, flattening off near the end.

For the cleared site there was a decrease in all factors; pH, EC, and cations, with depth. The lack of vegetation means that probably the main factor contributing to nutrient loss is leaching. There was a decreasing trend in cations for 0-5 and 75-85cm soil depths over the season, this decrease was similar in magnitude to that of the Acacia site. At 35-45cm there was an increase at the end of the season showing possible signs of accumulation, this did not occur under Acacia or fynbos vegetation.

3.5 Conclusions

The sandy soils studied were low in nutrients and differences were found between the three sites studied. The Fynbos site had lower nutrient contents and also had less leaching with depth than both of the other sites. Cations accumulated in the topsoil on all sites and the fynbos soil was least buffered indicated by pH fluctuations with little change in cations present. The cleared site experienced accumulation of cations within the profile studied, but rainfall and a low capacity for storage of nutrients by the soil probably resulted in nutrients being leached from the system with rapidity.

Alien invasion changed the nutrient status of the soil, increasing available nutrients and buffering. Removal of the alien legume at this point showed no significant nutrient differences in the soil compared to the still-invaded Acacia site. The cleared site may show more changes in the long term as decomposition progresses. Nutrients from initial decomposition will be the ones responsible for the changes seen in this study, however, with the following rainy season results would reflect a more long term situation regarding leaching and nutrient changes. It is expected that nutrient content and buffering capacity of the soil will increase. This would continue, with some leaching, if left undisturbed until decomposition is complete and as time progresses nutrients would be further leached from the soil. This is according to the three step process of decomposition as explained by Berg and Staaf, (1981) described in section 2.2.1.3. As a steady state with new vegetation is reached this process may change and decrease or prevent leaching of nutrients.

Chapter 4: Impact of the removal of an alien legume (*Acacia saligna*) on NO_X in a sandy soil in comparison to the natural fynbos and its effect on groundwater quality

4.1 Introduction

Yelenik et al., (2004) and Holmes, (2008) both found that there is more available N in the soil due to inputs by the Acacia than by natural fynbos vegetation. However these higher levels of N are likely to be short lived, particularly in such sandy soils (Witkowski, 1991a; Stock and Lewis, 1986). The N additions to these sandy soils as studied by Witkowski, (1991a) are not immobilized. Decomposition rates of fynbos vegetation are slow (Mitchell et al., 1986), and the decomposition of Acacia litter is faster (Witkowski, 1991a).

The Acacia uses more water than fynbos and causes a decrease in the flow of water in the fynbos biome (Holmes and Cowling, 1997). Leaching occurs more easily in the sandy soils studied (Pierzynski et al., 2005).The objectives of this study are to determine the effects of alien legume invasion and subsequent clearing on the NO_X content of the soils and also to assess the potential for groundwater pollution caused by leaching, due to vegetation removal.

4.2 Materials and methods

4.2.1 Site description

These studies were done at Riverlands Nature Reserve; the reserve is near Malmesbury, off the N7 about 60km out of Cape Town. A map with co-ordinates outlining the sites selected can be found in appendix A. The reserve was well suited to the study due to the presence of a long invaded and homogenous Acacia stand that was already scheduled for

clearing by Working for Water (WFW). The sandy soils and underground aquifer provided the appropriate site for sample selection so that soil and underground water could be studied according to the objectives. Riverlands Nature Reserve itself has mostly natural fynbos vegetation but much of the surrounding area is densely populated by the alien invader *Acacia saligna.* Three vegetation sites were used for this study. The natural fynbos vegetation was studied as a control, an alien invaded, Acacia, site on the neighbouring farm, and a site cleared of the alien invader during the study.

The site selected for study relating to fynbos vegetation was chosen because it is rich in diversity of species and represents natural fynbos vegetation. An advantage is that it is 11-15 years old where much of the surrounding fynbos is new since fires swept through in 2004 and 2005. The most common shrubs found on the site selected are of Proteaceae, Ericaceae and Rustaceae. *Euclea racemosa* and *Diospyros glabra* are also prevalent. Although the site is on a slope with a difference in elevation of under 0.5m, there is a difference in the vegetative community, roughly split into restio in the low-lying areas and protea in the high-lying areas. This is another reason why this site is a good representative of the general naturally growing fynbos community and specific studies of N within each individual species is not necessary to the findings of this study. This would, however, be interesting grounds for further study.

The Acacia site was separated from the fynbos site about 50 meters. Acacia trees were found in clusters with weedy grass and some herbaceous species in open patches, but these were sparse. Average canopy cover by the Acacia was 45%. Most of the ground was covered by Acacia litter. Trees varied in age varying from saplings to dead trees, but no seedlings were found. The land was used for cattle grazing until fairly recently before the study and this could explain the lack of seelings. Trees were in the range of 4 to 7 meters tall and most branches were infected with the parasitic gall *Uromycladium tepperianum* which was introduced as biological control.

Clearing was done on an Acacia site identical to the one described above. Acacia trees were cut down and left on the ground to imitate the practice most commonly used by farmers.

The underground aquifer below the soils studied was assumed to be multi- layered on the basis that water was found at a level of 0.9m below ground as well as at 18m below ground. A clay layer occurred at depths between 5 and 7 meters below the sandy soils of the nature reserve separating the two layers of underground water. This clay layer, seemingly continuous throughout the study area, acts as a nearly impermeable layer for the leaching of water, and water levels were found to be higher in the shallow aquifer. The flow of water was by and large from North-West to South-East (Jovanovic et al, 2008).

The climate is Mediterranean with an average rainfall of approximately 440mm per year, most of this occurring between May and August. Although during the rainy season water is seen to accumulate on the soil surface of the lower lying areas. For the most part evaporation is more than rainfall. Summers are warm with a mean daily temperature of 27.9°C in February, compared to 7°C in July.

4.2.2 Experimental design

Soil samples were taken every fortnight from May to November 2007 at approximately the same time of day (afternoons between 2 and 5) and once more in early December 2007. Samples were taken using a Jarrett soil auger and three replicate samples were taken per site, per sampling date at three soil depths; 0-5cm, 35-45cm and 75-85cm. The replicates were not analysed individually, but rather averaged physically by mixing. As a result the analysed sample represented three sampling points for each depth increment of every test area. To determine the validity of physical averaging a separate experiment was undertaken. All three replicates were analysed separately on one of the sampling sessions and the results were averaged statistically for comparison with physically

averaged samples. These composite samples were seen to correspond well to the statistically averaged samples.

Samples were collected along the slope, in an approximately north to southerly direction for the fynbos site and South to Northerly direction for the Acacia and cleared sites. Examples of each site of vegetation were not replicated because the soil samples within the sites were replicated. This resulted in a pseudoreplicate design, although emphasis was placed on the differences between sites, soil samples within each site were replicated. Unfortunately this has prevented the use of extensive statistical testing.

Soil samples were stored wet and under cool conditions before being air dried, sieved and analysed. Samples were incubated so that N concentrations may stabilise. Sieving and improved air circulation may increase mineralisation in stored soil samples (Nordmeyer and Richter, 1985), in this way the maximum mineralised N can be determined and this is of relevance to determine the potential groundwater pollution. In this way the mineralised N may be overestimated, but if the mineralised N had been underestimated, that would be a problem for this study, resulting in lower estimated levels of N for leaching to groundwater. The difference between the maximum mineralised N and mineralised N before storage is expected to be insignificant because of the total N that is so much higher.

Groundwater samples were collected from a series of boreholes, four pairs; one deep and one shallow, as well as an additional five shallow boreholes. The deep boreholes were drilled 8m into the clay layer below the sand; shallow boreholes were drilled to the top of this clay layer. The placement of the five shallow boreholes was determined according to the direction of the groundwater flow, for this six additional well points, at the shallow depth, were augured (Jovanovic, et al., 2008).

4.2.3 Soil chemical analyses

Soils were air dried and sieved through a 2mm sieve. pH was measured in distilled water and in 1M KCl from a 1:2.5 solution and EC was measured in a 1:5 soil water solution. pH and EC were determined for all samples, replicates and dates separately, according to the methods outlined in Mc Lean, (1982) and Rhoades, (1982). Total carbon and nitrogen content as well as the levels of basic cations, Ca, Mg, Na and K were determined for three selected dates. Due to financial constraints and the level of relevance for the study they were not analysed for each sampling date. The selected sampling dates were; pre clearing (8 May 2007), soon after clearing (24 June 2007) and at the end of the sampling season (9 December 2007). Total carbon and nitrogen were determined by combustion (gas chromatography) as in the method outlined by Bremner and Blackmer, (1982) and the cations were measured from a 1:4 soil NH_4OAc extract (as in the method described by Rowell (1995)), and analysed by atomic absorption spectroscopy (Thomas, 1982; Knudsen et al., 1982).

Once it was checked that composite samples were true representatives of the average of the replicates, the samples were used for the analysis of anions. Composites of the three replicates were made from a 1:5 soil water extract. This was done for each sampling date and soil depth, and then used for anion determination by ion chromatography (Rhoades, 1982). Raw data is tabulated in Appendix C.

4.2.4 Groundwater analysis

Groundwater samples were collected and analysed as described in Greenberg, (1992). Groundwater levels and temperature were monitored twice a week using a levelogger. Hydraulic conductivity was determined using the slug test method with the software AQUIFER TEST Pro 3.5. Monthly measurements of Ca, Mg, ammonia as N mg/l, NOx as N mg/l, SO_4, Cl, $CaCo_3$, Fe, DOC, EC, Kjeldahl N and pH (all the above in mg/l) were taken and analysed at CSIR, these were reported in Jovanovic et al., (2008).

4.3 Results

4.3.1 C:N ratio

Figure 4-1 a, b and c show the change in C:N ratio over time, separated by depth. At 0-5cm there is a decrease over the sampling period and the trend under Fynbos differs from the other sites, with an initial increase rather than decrease.

At soil depths of 35-45 and 75-85cm, there is an increase in C:N ratio on all sites. The initial increase at 35-45cm on the Acacia and cleared sites are larger than for the fynbos site, but the end of season values are similar. C:N ratios do not differ much over the different sites at the end of the season, but fynbos has a consistently lower C:N ratio than the other two sites.

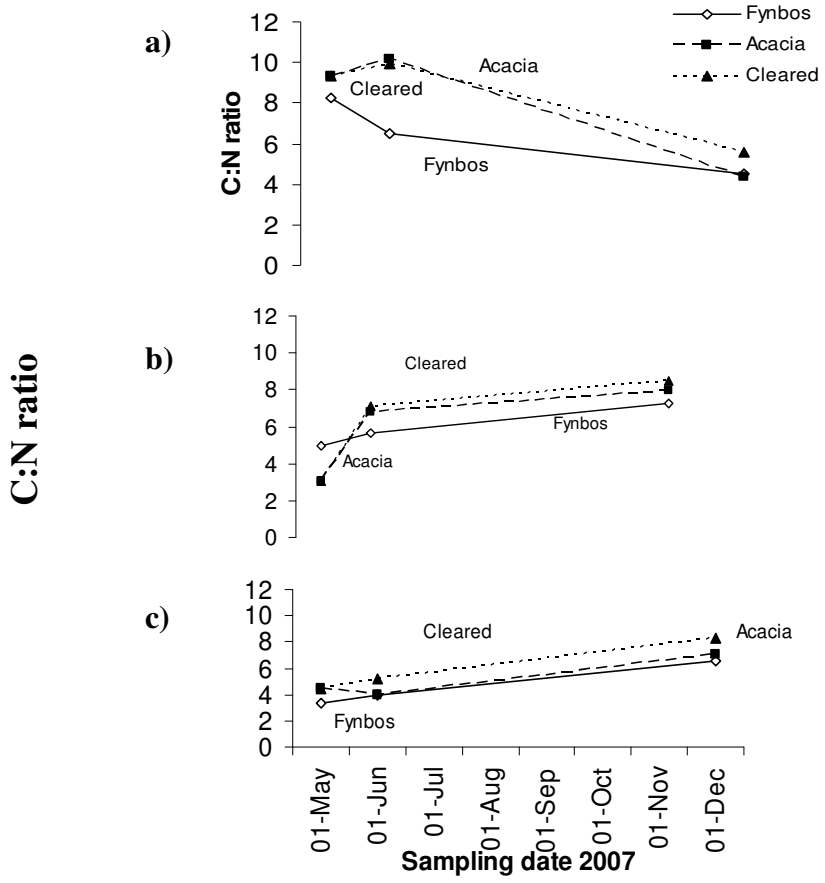


Figure 4- 1: Total C:N ratio over the sampling period (2007) at soil depths a) 0-5cm b) 35-45cm, c) 75-85cm

4.3.2 Composite sample testing

Figure 4- 2 shows the difference between the soils analysed for NO_x after physical mixing in the lab against the results averaged once all replicates had been analysed. The difference is small, less than 3mg/l in all cases.

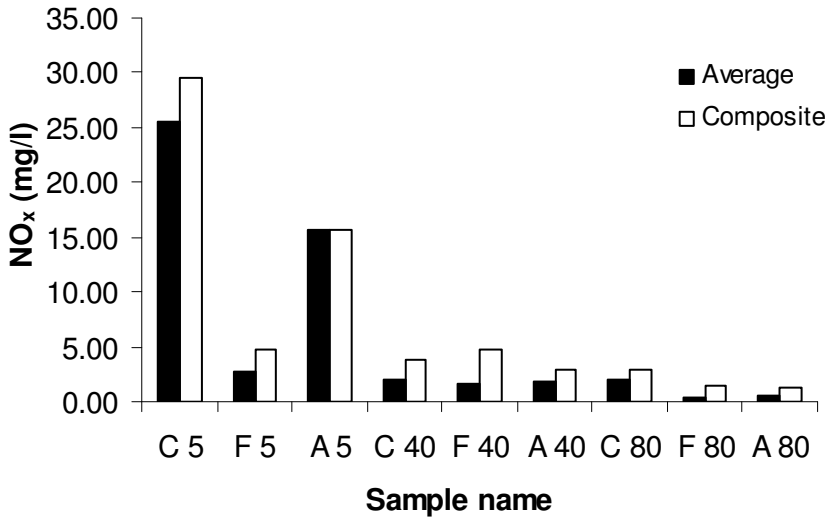


Figure 4- 2: Results for NO_x of Soil samples physically averaged in the lab (labled composite) plotted against the results for those statistically averaged. Samples were labled with a letter depicting their site origin (C: cleared, F: fynbos and A: Acacia) and a number representing the average depth of sampling in cm

4.3.3 Nitrate and nitrite fluxes

The NO_X (NO_2 +NO_3) levels at 0-5cm are higher than at 35-45 and 75-85cm (Figure 4- 3 a, b and c). Trendlines have been drawn to make understanding of the general trend over the season clearer, these are not regression lines and therefore have no R^2 or equation shown on the graph. At all soil depths the fynbos site had lower N values. There are a few NO_X peaks during the season, one on the cleared site seen on June 24 at 0-5cm. Another peak that is consistent throughout the sites and soil depths is on September 28. This peak does not occur on the fynbos site at 0-5cm, but can be seen at the lower soil depths. Most

soil NO_X is present in the top 0-5cm of soil, with a decrease with depth until a great deal less is found at 75-85cm.

At a depth of 0-5cm, there is a strong decreasing trend in NO_X as the season progresses. The least change was observed under fynbos vegetation. The cleared site experiences a rapid decrease in NO_X during the season with very little left at the end of the sampling period. Under Acacia, the decrease continues steadily, but slightly less steeply than the cleared site.

The cleared site again has a rapid decrease in NO_X at 35-45cm, but at 75-85cm, there is far less change than at the shallower soil depths. The trendline of the Acacia curve shows a consistent decrease, and the absolute values are only slightly higher than on the cleared site. Although the difference is small, a larger seasonal decrease was shown for the Acacia site than the cleared site at a depth of 75-85cm. There is an increase in NO_X as the season progresses on the fynbos site at soil depths 35-45 and 75-85cm.

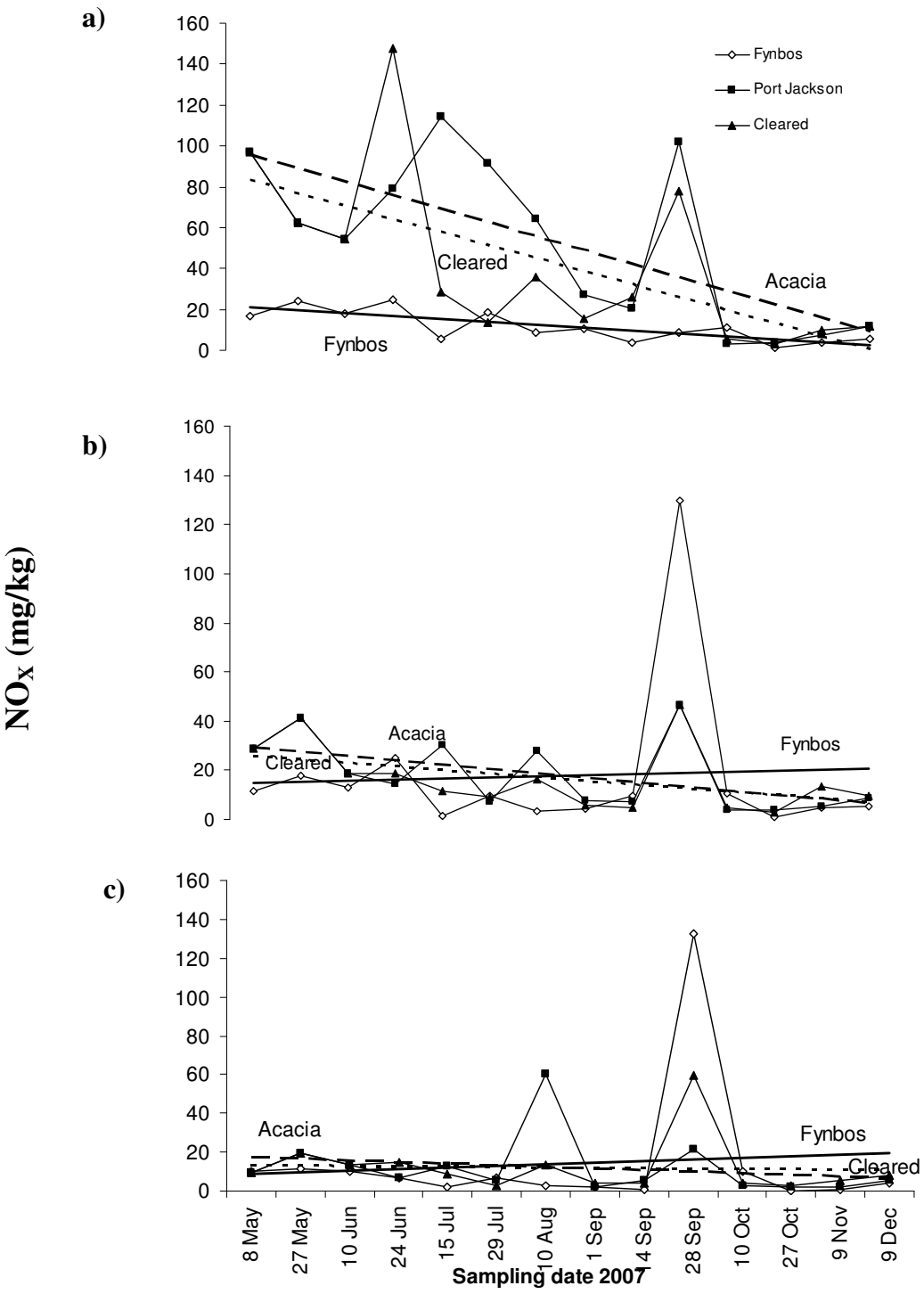


Figure 4- 3: Seasonal changes in NO_X (mg/kg) under the three sampling sites at soil depths a) 0-5cm, b) 35-45cm and c) 75-85cm with linear trendlines added to show the general trend over the season

4.3.3 Depth differences

Figure 4-4 a, b and c represent N in NO_X (mg/l) averaged over the entire sampling period as a function of depth, groundwater included. Error bars (standard deviation, based on variation between sampling dates) are given. All NO_x values were initially determined in mg/l but had to be converted to N in NO_x to be compared with the groundwater for the purpose of determining potential pollution. The N in NO_x reported for groundwater were analysed from the shallow boreholes only, the values represent the average of four water samples. NO_x decreases with depth under Acacia and on the cleared site. These two sites have higher groundwater N than in the soil and than under fynbos vegetation. The changes in N with depth in soil and into the groundwater, under fynbos are much smaller.

There is a vast difference in magnitude between the changes of the fynbos site and the two others. There is a far lower N content under fynbos vegetation in soil and water. The difference in N in NO_x between the cleared and Acacia sites is not noticeable according to Figure 4-4.

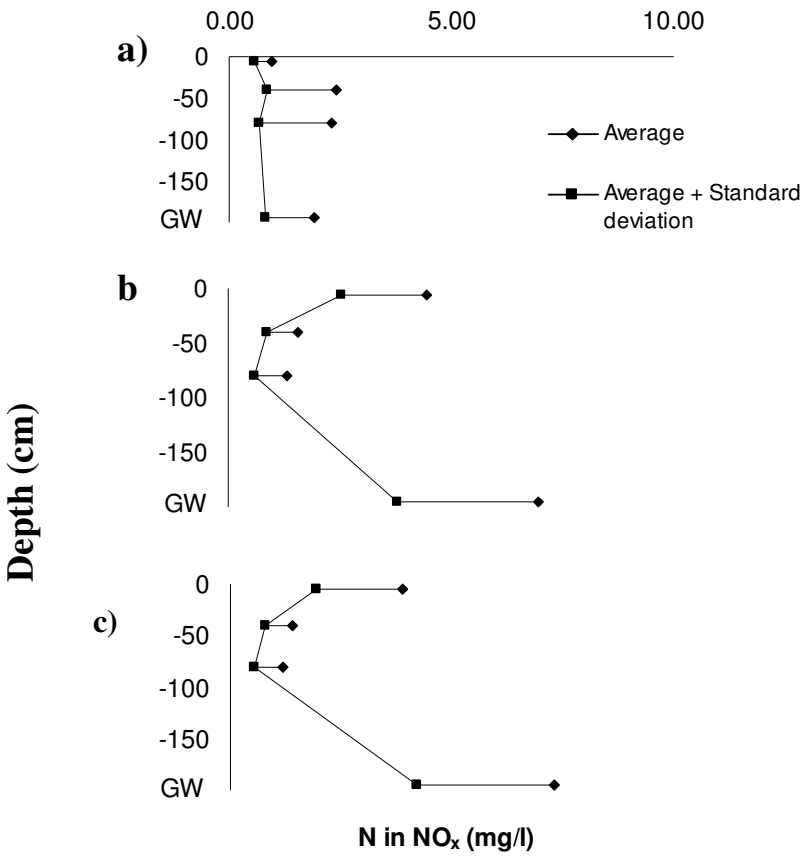


Figure 4- 4: Change in N in NO_X (mg/l) averaged over the sampling period (2007) with depth and into groundwater showing +1 standard deviation on the a) Fynbos, b) Acacia and c) Cleared site

4.3.4 Soil and groundwater modelling

Modelling was done using HYDRUS 2D for the unsaturated zone and Visual MODFLOW for the saturated zone. Soil N analysis was used in HYDRUS 2D and the results predicting recharge and nitrogen leaching were again used in Visual MODFLOW for the prediction of concentration of NO_3^- in groundwater (Jovanovic et al., 2008).

Figure 4-5 tracks the changes in N in NO_x in groundwater over the sampling season. Initially the Acacia (uncleared) site had the highest N concentration, but after July the cleared site had the highest N concentrations. The fynbos site had consistently low N concentrations, with a small peak between July and August.

Appendix D shows the spatial distribution of N in groundwater as predicted by Visual MODFLOW. The cleared site stands out as having a higher content of N than the other sites and than the surrounds.

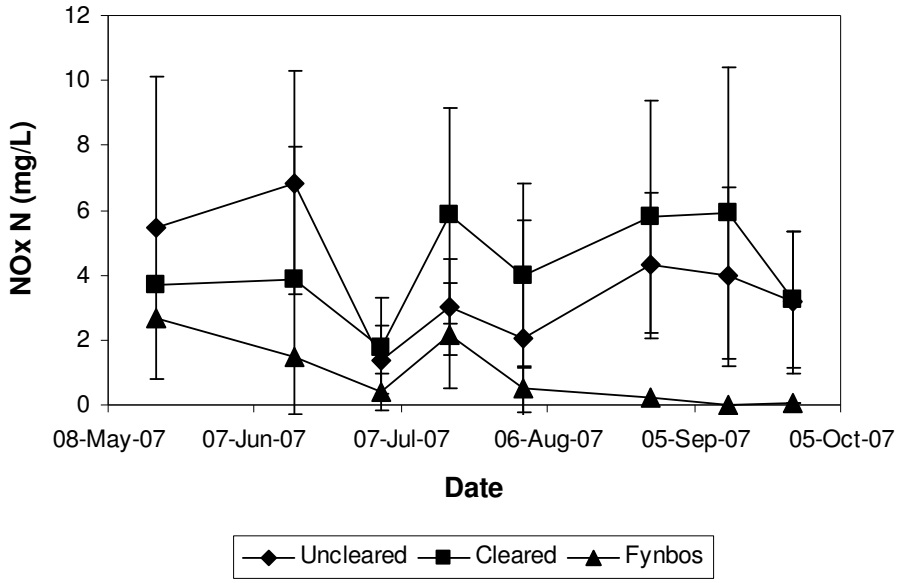


Figure 4- 5: N in NO_x in groundwater for the three study sites over a sampling period from May to October (Jovanovic et al., 2008)

4.4 Discussion

At a depth of 0-5cm, the most sensitive depth to temporal changes and microbial activity, the C:N ratio decreases over the sampling period, indicating the decomposition that takes place over this time period. Decomposition progression with time is expected, although the wet season at the beginning of sampling will hinder decomposition due to soil wetness and low microbial activity. When temperatures increase and the soil reaches a more favorable oxygen content for decomposition and microbial activity, decomposition rates will increase, which will decrease the C:N ratio (Knorr et al., 2005). The total C and N also decline over the sampling period due to progressive mineralisation.

The increase in the C:N ratio at 35-45 and 75-85cm is due to an accumulation of organic N to a larger degree than of organic C (if any). The cause of this is that the breakdown products of plant litter are soluble and will be leached downward through the profile. Partially decomposed N is more soluble than decomposing C. N levels in invaded areas can be so high that it has been suggested to add a mulch to decrease the C:N ratio by immobilizing N (Yelenik, 2004 and Holmes, 2008). In a study done by Witkowski (1991a), it was found that the Acacia produces not only more plant litter, but N rich litter, adding three times the amount of N compared to the natural fynbos. Decomposition of fynbos litter also takes longer than decomposition of the Acacia litter (Witkowski, 1991a).

The higher NO_X levels at a depth of 0-5cm for all sites are due to the accumulation and decomposition of organic matter on the soil surface and subsequent leaching. Higher levels of N were seen on the Acacia site compared to the fynbos site, particularly at a depth of 0-5cm. The C:N ratio was considered a good indicator used for the prediction of decomposition, but Mafongoya et al (1998) have said that this is not the case with most agroforestry systems. Legumes release more N into the soil than other plants because of their high N contents. Another factor contributing to the higher N content of soils under legume vegetation is that the soil is not depleted of N as much because N from the atmosphere is utilized during plant growth (Papstylianou, 1988). Yelenik et al., (2004) found that after the invasion of fynbos by *Acacia saligna* there was an increase in available NO_3 and NH_4^+. Witkowski (1991a) found that the N content in fynbos ecosystems increased both in the litter and in the soil after invasion by the Acacia.

Clearing occurred on the 18th June; the values for the first three sampling dates (the dates preceding clearing, where both sites had Acacia) on the cleared site are the same as for the Acacia site. The peak under the cleared site at June 24th is due to the sudden input of nutrients from the cleared plant litter along with rainfall. The sharp increase in NO_X on 28 September is, most likely, due to the temperature increase and the subsequent decrease in soil water content at the beginning of spring which causes the soil microbes to become active and begin to oxidize N.

The slopes of the graphs showing anion and NO_x change over the sampling season can be seen in Figure 4-3. Gradients for changes in NO_x were calculated from the trendlines in Figure 4- 3 and the same was done for anions. These gradients are plotted in Figure 4- 6. A steeper slope indicates a faster decrease in anions or NO_X. The Acacia site loses NO_X at the highest rate and the cleared site at a rate almost as high. On the fynbos site N is lost from topsoil and is intercepted and accumulated in the subsoil. There is a flattening of the slope under Acacia and on the cleared site at 35-45 and 75-85cm this is due to a decrease in loss of NO_X as the season progresses. The slopes of NO_x and anion changes are similar in value and trend for each site during the sampling period.

The anion loss indicates overall leaching. The loss of anions is higher than the NO_X loss for all sites and all soil depths because NO_X is intercepted. Although the loss decreases with depth there is no accumulation at the lower soil depths. Leaching levels at the lower soil depths is less for all sites. Leaching under fynbos is less than leaching on the other two sites. NO_X is not moving through the profile at a faster rate than the total anions.

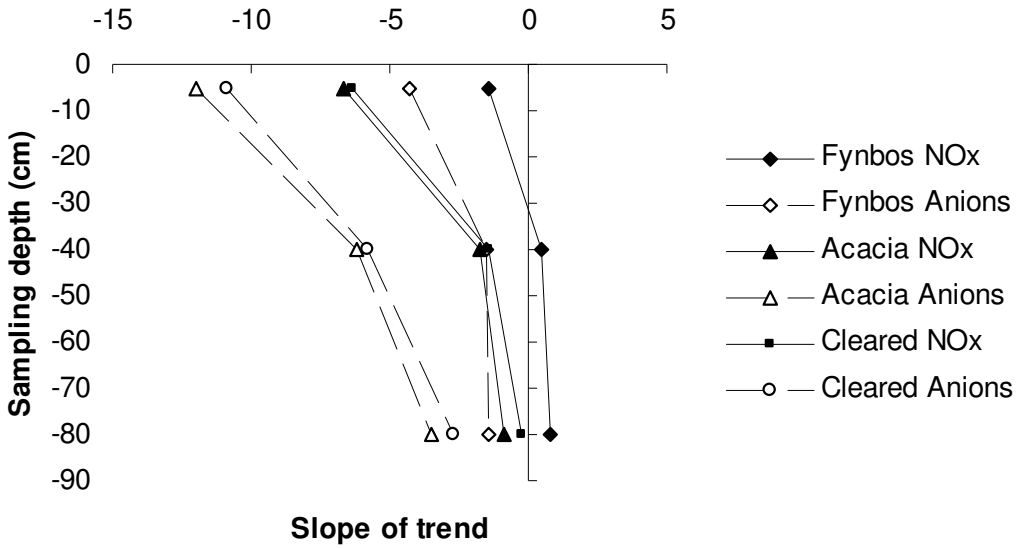


Figure 4- 6: Slopes of the change in Anions and NO_X at the three different soil depths on the three study sites

In general the soil NO_X content decreases over the winter season, there is also a decrease in total NO_X per treatment with depth. The rapid decrease on the cleared site is a result of high leaching, because there is only minimal plant uptake or inputs beyond the surface litter. Leaching and plant uptake are the reason for the decrease on the Acacia site as well as on the fynbos site.

The decrease of N in NO_X with depth after invasion, is because decomposition takes place on the surface and leaching to groundwater occurs quickly in the sandy soil. In the groundwater concentrations were higher. Due to the removal of an N source and the high organic N input from plant removal the cleared site has more NO_X within the profile than the Acacia site, but at this point the difference is small. Appendix D contains a map depicting the leaching occurring on each site separately, values are safely below the average 10mg/l required for pollution, however higher N in the groundwater is predicted under the cleared site.

4.5 Conclusions

There is a clear increase in soil nutrients, N and NO_X in this case, on the Acacia and cleared sites in comparison to the control, fynbos site due to invasion of this leguminous invader. The N in groundwater for the fynbos site is also far lower than the other two sites.

The site invaded by *Acacia saligna* shows an increase in the N content of the ecosystem. Once the alien invader has been removed the N in organic litter is decomposed and leached through the sandy soils and into the groundwater. The NO_X levels in the groundwater 6 months after clearing are not sufficient for water pollution, the highest value reported being 12mg/l. This was found under the Acacia site, but had dropped to a value of 4mg/l within 2 weeks. The highest averaged value during the study season was found under the cleared site at 4.34 mg/l. The Acacia site N concentration was not much

lower with 3.78mg/l, both of these are far from a threat to the strictest limits placed on N at 10mg/l.

After the cutting down of the Acacia in the area, the water in the system should increase due to lower demand from plants. This litter left will increase the inputs of N, and lack of vegetation along with increased amounts of water may cause these sandy soils to be depleted of nutrients. The increase of water leaching through the profile to the groundwater will result in dilution of groundwater and has the potential to redistribute the NO_X areas where it could be of benefit.

The final short term outcome- being an increase or decrease in N concentration in groundwater- is difficult to predict without being able to quantify all these parameters in such a short study, and this study site should be monitored for an indication of more long term response.

Chapter 5: Conclusions

The objectives of this study were to determine the differences in soil nutrients, focusing on nitrate and nitrite levels, between a site of natural fynbos vegetation, a site invaded by a leguminous alien invader (*Acacia saligna*) and a site cleared of the alien legume. Another aim was to study the effects this could have on groundwater with regards to N contamination due to organic matter breakdown and nitrate and nitrite leaching. The effect of adjusted N levels on the re-establishment of fynbos vegetation was considered in the literature review. To achieve the objectives it was necessary to study the general soil nutrient status on each site and relate this to the season's rainfall and groundwater N levels and then compare these parameters for each site.

Soil nutrient status on the natural fynbos site was lower than on the other sites. Less seasonal fluctuation of anions and N was seen, these soils were not as well buffered and also had lower organic matter contents. There was also less change in anions, NO_X and EC compared to the other sites. The invaded site had higher nutrient contents with larger seasonal variation and a better buffering capacity due to the higher organic matter content and higher N-rich organic matter. The long term re-establishment of fynbos is not likely to be hindered by the increased N content even though the soils are now better suited to grasslands, since N is not stored in the soil for long periods of time and rate of leaching in these sandy soils is high.

Removal of the alien legume did not result in large nutrient changes, but after clearing there was an increase in nutrient availability due to the input of large amounts of organic litter. This was amplified by organic matter breakdown and leaching with rainfall. N in NO_X in groundwater was highest for the cleared site, but not by much and through the soil profile there was more NO_X under the Acacia site. The average groundwater levels of N in NO_X are less than 5mg/l for all sites, this falls into the acceptable N levels for all uses and cannot be called pollution. It is safe to say that one season after the removal of the Acacia there was no groundwater pollution. It is still necessary to point out that the organic litter inputs due to clearing of the legume with this method, where the litter is left

on the surface, and the removal of such a high water utilising plant could increase the soluble N in groundwater in the near future. Further study is needed to do a full assessment of the long term effects on groundwater due to alien legume removal.

As can be seen there is great scope for further study, long term changes in soil and groundwater N can be measured. This should probably include other forms of N, most importantly NH_4^+. Since this study was aimed at the effect of leaching of N into groundwater and no specific N leaching experiments were done, it would be interesting to investigate the process of leaching on these soils.

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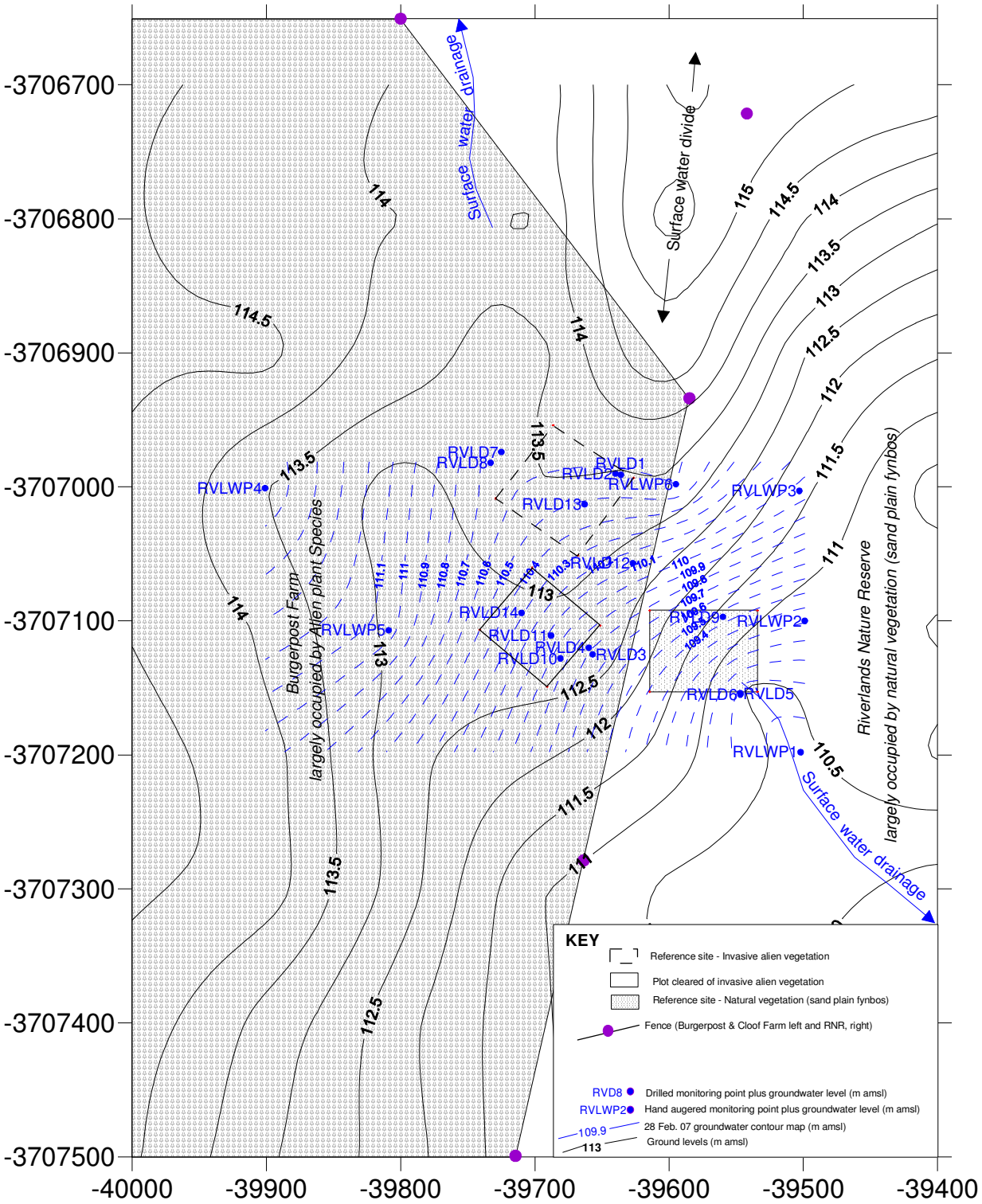
APPENDICES

Appendix A- Site layout including borehole placement and GPS co-ordinates (Jovanovic et al, 2008)



Appendix B – Soil descriptions

A representative soil from the fynbos site: Lamotte

Sample	Horizon	Depth (cm)	Soil Description	Site description	Photo
A	Orthic A	0-20	Dry; light yellow-brown (2.5Y6/3); fine sand; weak structure, apedal; loose in the dry and moist state, non-sticky and non-plastic when wet	Natural fynbos vegetation, semi-arid region, convex footslope, slope about 1%, many large pores and root canals in the topsoil, surrounded by organic matter. Less than 1% clay.	
E	E	20-60	Dry-moist; mottled grey; fine sand; weak structure, apedal; loose in the dry and moist state, non-sticky and non-plastic when wet		
B	Podzol B	60+	Slightly moist; non-uniform yellow (10YB7/6); fine sand; weak structure, apedal; loose in the dry and moist state, non-sticky and non-plastic when wet		

Summary of the soils of the Acacia and cleared sites: Vilafontes or Constantia

Sample	Horizon	Depth (cm)	Soil Description	Site description	Photo
A	Orthic A	0-30	Dry; light yellow-brown; fine sand; weak structure, apedal; loose in the dry and moist state, non-sticky and non-plastic when wet	Acacia and cleared land used for cattle grazing, semi-arid region, many large pores and root canals in the topsoil, surrounded by organic matter. Clay of just over 1% and less.	
E	E	30-90	Dry-moist; mottled grey; fine sand; weak structure, apedal; loose in the dry and moist state, non-sticky and non-plastic when wet		
B	Neocutanic B, or yellow brown apedal B	90+	Slightly moist; uniform and non-uniform yellow to yellow-brown ; fine sand; weak structure, apedal; loose in the dry and moist state, non-sticky and non-plastic when wet		

Appendix C – Raw data

(Results have been converted from mg/l to mg/kg)

Sample details				pH		Ec	Basic cations (mg/kg)				C:N	
Date	Site	Depth	Rep	water	KCl	µS/cm	Ca	Mg	Na	K	C	N
8 May	Fynbos	5	1	5.31	4.12	25.60	98.00	18.80	42.80	15.60	0.67	0.10
8 May	Fynbos	40	1	5.61	4.43	16.66	60.00	16.40	37.20	12.40	0.36	0.07
8 May	Fynbos	80	1	5.38	4.55	12.00	34.40	13.20	38.00	13.60	0.14	0.04
8 May	Fynbos	5	2	6.00	4.10	20.10	100.80	17.60	41.60	26.80	0.56	0.05
8 May	Fynbos	40	2	6.10	4.45	15.68	64.00	15.60	42.80	19.20	0.32	0.05
8 May	Fynbos	80	2	5.37	4.67	30.00	28.80	12.80	37.60	72.80	0.17	0.04
8 May	Fynbos	5	3	5.34	4.44	18.61	92.80	15.60	30.80	27.60	0.42	0.05
8 May	Fynbos	40	3	5.38	4.39	11.07	38.00	11.60	25.60	16.80	0.17	0.05
8 May	Fynbos	80	3	5.62	4.78	6.69	28.40	8.40	38.80	14.40	0.12	0.05
8 May	Acacia	5	1	5.31	4.51	53.40	196.00	73.20	62.00	28.80	0.87	0.08
8 May	Acacia	40	1	5.45	4.24	20.20	56.00	17.20	48.80	15.60	0.37	0.05
8 May	Acacia	80	1	5.54	4.43	20.90	23.20	7.20	56.80	15.60	0.26	0.05
8 May	Acacia	5	2	5.66	4.68	36.10	78.00	13.20	40.40	24.00	0.29	0.05
8 May	Acacia	40	2	5.26	4.52	22.50	74.40	8.40	30.40	4.80	0.18	0.05
8 May	Acacia	80	2	5.56	4.69	6.48	23.60	3.20	33.20	14.80	0.16	0.04
8 May	Acacia	5	3	5.71	5.01	53.50	240.80	53.60	29.60	35.60	0.80	0.08
8 May	Acacia	40	3	5.43	4.69	24.10	64.00	15.20	44.00	10.80	0.28	0.17
8 May	Acacia	80	3	5.13	4.61	19.63	24.80	11.20	38.00	18.00	0.11	0.03
8 May	Cleared	5	1	5.31	4.51	53.40	196.00	73.20	62.00	28.80	0.87	0.08
8 May	Cleared	40	1	5.45	4.24	20.20	56.00	17.20	48.80	15.60	0.37	0.05
8 May	Cleared	80	1	5.54	4.43	20.90	23.20	7.20	56.80	15.60	0.26	0.05
8 May	Cleared	5	2	5.66	4.68	36.10	78.00	13.20	40.40	24.00	0.29	0.05
8 May	Cleared	40	2	5.26	4.52	22.50	74.40	8.40	30.40	4.80	0.18	0.05
8 May	Cleared	80	2	5.56	4.69	6.48	23.60	3.20	33.20	14.80	0.16	0.04
8 May	Cleared	5	3	5.71	5.01	53.50	240.80	53.60	29.60	35.60	0.80	0.08
8 May	Cleared	40	3	5.43	4.69	24.10	64.00	15.20	44.00	10.80	0.28	0.17
8 May	Cleared	80	3	5.13	4.61	19.63	24.80	11.20	38.00	18.00	0.11	0.03
24 Jun	Fynbos	5	1	5.43	4.39	17.50	58.00	9.20	28.80	10.80	0.31	0.05
24 Jun	Fynbos	40	1	5.31	4.69	5.72	36.80	6.80	4.80	6.00	0.14	0.03
24 Jun	Fynbos	80	1	5.64	4.82	4.73	29.20	7.20	29.20	10.00	0.13	0.04
24 Jun	Fynbos	5	2	5.20	4.45	18.35	51.60	6.80	6.80	9.20	0.29	0.05

Sample details				pH		Ec	Basic cations (mg/kg)				C:N	
Date	Site	Depth	Rep	water	KCl	µS/cm	Ca	Mg	Na	K	C	N
24 Jun	Fynbos	40	2	5.09	4.51	7.17	35.60	6.40	5.20	8.00	0.25	0.04
24 Jun	Fynbos	80	2	5.64	4.71	8.12	19.20	6.40	6.00	5.20	0.20	0.04
24 Jun	Fynbos	5	3	5.32	4.44	11.03	75.60	9.60	8.00	12.80	0.38	0.05
24 Jun	Fynbos	40	3	5.46	4.57	9.24	67.20	14.80	6.00	11.20	0.29	0.05
24 Jun	Fynbos	80	3	5.43	4.76	4.46	22.40	8.40	4.80	6.00	0.15	0.04
24 Jun	Acacia	5	1	5.38	4.70	52.70	114.00	33.60	29.20	20.80	0.55	0.07
24 Jun	Acacia	40	1	5.67	4.26	22.30	17.60	8.40	40.80	8.00	0.27	0.04
24 Jun	Acacia	80	1	5.16	4.37	26.90	8.00	4.00	33.60	9.60	0.18	0.04
24 Jun	Acacia	5	2	5.76	5.02	30.50	228.40	46.80	34.00	15.60	0.93	0.08
24 Jun	Acacia	40	2	5.46	4.68	11.22	79.60	20.40	27.60	8.00	0.26	0.04
24 Jun	Acacia	80	2	5.02	4.78	5.16	11.60	5.20	6.00	5.60	0.11	0.03
24 Jun	Acacia	5	3	5.26	4.41	39.40	228.80	45.20	34.40	20.80	0.86	0.08
24 Jun	Acacia	40	3	5.41	4.41	11.85	22.00	13.20	16.40	9.60	0.29	0.04
24 Jun	Acacia	80	3	5.39	4.78	5.04	6.40	3.20	7.60	4.00	0.11	0.03
24 Jun	Cleared	5	1	5.24	4.53	85.10	183.20	23.60	19.20	22.00	0.50	0.06
24 Jun	Cleared	40	1	5.51	4.60	12.01	59.60	7.20	8.80	5.20	0.24	0.03
24 Jun	Cleared	80	1	5.56	4.72	9.16	19.20	4.00	30.80	41.60	0.17	0.03
24 Jun	Cleared	5	2	5.46	5.10	56.20	192.00	30.00	23.20	28.80	0.41	0.04
24 Jun	Cleared	40	2	5.59	4.45	20.70	44.40	8.40	56.40	7.20	0.29	0.04
24 Jun	Cleared	80	2	5.45	4.73	11.78	22.80	4.00	32.00	57.20	0.13	0.03
24 Jun	Cleared	5	3	5.53	4.68	98.70	116.80	26.40	33.20	60.80	0.68	0.06
24 Jun	Cleared	40	3	4.95	4.27	20.30	57.60	11.60	33.60	60.00	0.25	0.04
24 Jun	Cleared	80	3	5.12	4.58	13.71	22.80	6.00	11.20	12.80	0.17	0.03
9 Dec	Fynbos	5	1	5.91	4.91	8.42	53.60	6.80	27.20	9.20	0.45	0.06
9 Dec	Fynbos	40	1	5.91	4.19	5.10	39.60	6.80	25.20	6.80	0.65	0.08
9 Dec	Fynbos	80	1	5.83	4.43	6.18	22.00	6.40	26.00	6.80	0.78	0.11
9 Dec	Fynbos	5	2	5.71	4.64	11.18	114.80	15.20	28.40	9.60	0.42	0.12
9 Dec	Fynbos	40	2	5.30	4.38	11.68	96.00	14.00	28.80	8.80	0.47	0.06
9 Dec	Fynbos	80	2	5.54	4.55	10.26	18.00	10.00	34.80	4.00	0.24	0.05
9 Dec	Fynbos	5	3	5.70	4.58	6.98	57.20	13.20	30.80	10.40	0.16	0.05
9 Dec	Fynbos	40	3	6.42	4.33	11.37	37.60	9.20	30.00	6.40	0.33	0.06
9 Dec	Fynbos	80	3	5.87	4.59	13.11	11.20	4.80	30.40	4.00	0.41	0.06
9 Dec	Acacia	5	1	5.26	4.57	5.80	77.20	11.20	28.80	7.60	0.24	0.05
9 Dec	Acacia	40	1	5.44	4.61	8.53	58.00	8.80	29.20	4.80	0.37	0.05
9 Dec	Acacia	80	1	5.59	4.54	9.02	13.60	2.80	26.40	2.80	0.25	0.05

Sample details				pH		Ec	Basic cations (mg/kg)				C:N	
Date	Site	Depth	Rep	water	KCl	µS/cm	Ca	Mg	Na	K	C	N
9 Dec	Acacia	5	2	5.46	4.59	7.14	57.20	10.80	30.40	5.60	0.13	0.04
9 Dec	Acacia	40	2	5.19	4.32	8.77	29.20	6.80	20.40	3.60	0.51	0.06
9 Dec	Acacia	80	2	4.87	4.26	5.08	8.80	2.00	20.80	2.80	0.48	0.05
9 Dec	Acacia	5	3	6.83	4.25	13.85	254.80	24.40	27.20	14.80	0.24	0.05
9 Dec	Acacia	40	3	6.03	4.66	11.50	88.40	11.60	30.80	7.60	0.31	0.04
9 Dec	Acacia	80	3	5.41	4.93	5.41	42.40	6.00	26.00	3.60	0.33	0.05
9 Dec	Cleared	5	1	5.70	4.55	10.86	114.00	9.60	27.20	4.80	0.18	0.04
9 Dec	Cleared	40	1	5.99	4.62	11.15	134.00	10.00	30.80	7.20	0.38	0.05
9 Dec	Cleared	80	1	6.02	4.81	10.79	38.80	4.80	37.60	4.00	0.30	0.04
9 Dec	Cleared	5	2	5.24	5.11	8.07	53.60	5.20	23.60	6.00	0.15	0.03
9 Dec	Cleared	40	2	5.49	4.38	5.89	48.00	4.00	21.60	5.20	0.47	0.05
9 Dec	Cleared	80	2	5.90	4.45	4.59	52.40	4.00	23.60	6.80	0.36	0.04
9 Dec	Cleared	5	3	6.41	4.86	8.04	310.00	18.40	27.60	7.60	0.28	0.04
9 Dec	Cleared	40	3	6.11	5.20	11.57	196.00	12.80	27.60	5.60	0.00	0.00
9 Dec	Cleared	80	3	6.16	4.93	6.32	41.20	6.00	27.20	3.20	0.00	0.00

Sample details			pH		Ec	Anions (mg/kg)				
Date	Site	Depth	water	KCl	µS/cm	Cl	NO_2^-	NO_3^-	SO_4^{2-}	∑
8 May	Fynbos	5	5.31	4.12	25.60	18.95	16.95	0.00	15.50	51.40
8 May	Fynbos	40	5.61	4.43	16.66	18.60	11.55	0.00	12.90	43.05
8 May	Fynbos	80	5.38	4.55	12.00	32.90	0.00	9.85	15.20	57.95
8 May	Acacia	5	5.31	4.51	53.40	28.90	1.40	95.75	22.15	148.20
8 May	Acacia	40	5.45	4.24	20.20	26.30	0.00	28.80	17.40	72.50
8 May	Acacia	80	5.54	4.43	20.90	26.40	0.00	9.55	13.95	49.90
8 May	Cleared	5	5.31	4.51	53.40	28.90	1.40	95.75	22.15	148.20
8 May	Cleared	40	5.45	4.24	20.20	26.30	0.00	28.80	17.40	72.50
8 May	Cleared	80	5.54	4.43	20.90	26.40	0.00	9.55	13.95	49.90
27 May	Fynbos	5	5.25	4.46	16.05	16.35	1.00	23.15	13.15	53.65
27 May	Fynbos	40	5.64	4.71	10.23	16.40	0.00	18.00	12.55	46.95
27 May	Fynbos	80	6.00	5.07	5.66	15.35	0.00	11.05	11.80	38.20
27 May	Acacia	5	5.69	4.75	31.80	26.45	0.00	62.25	18.80	107.50
27 May	Acacia	40	5.65	4.69	23.10	23.70	0.00	41.55	13.15	78.40
27 May	Acacia	80	5.52	4.84	16.05	22.05	0.00	19.55	13.15	54.75
27 May	Cleared	5	5.69	4.75	31.80	26.45	0.00	62.25	18.80	107.50
27 May	Cleared	40	5.65	4.69	23.10	23.70	0.00	41.55	13.15	78.40
27 May	Cleared	80	5.52	4.84	16.05	22.05	0.00	19.55	13.15	54.75
10 Jun	Fynbos	5	5.31	4.31	14.93	12.30	0.00	17.90	3.75	33.95
10 Jun	Fynbos	40	5.42	4.77	15.65	19.40	0.00	13.15	15.55	48.10
10 Jun	Fynbos	80	6.35	5.04	4.71	18.70	0.00	10.30	13.85	42.85
10 Jun	Acacia	5	5.60	4.74	31.70	15.95	0.00	54.65	15.00	85.60
10 Jun	Acacia	40	5.90	4.82	18.12	16.50	0.00	18.75	13.70	48.95
10 Jun	Acacia	80	5.38	4.48	8.80	14.80	0.00	13.65	13.20	41.65
10 Jun	Cleared	5	5.60	4.74	31.70	15.95	0.00	54.65	15.00	85.60
10 Jun	Cleared	40	5.90	4.82	18.12	16.50	0.00	18.75	13.70	48.95
10 Jun	Cleared	80	5.38	4.48	8.80	14.80	0.00	13.65	13.20	41.65
24 Jun	Fynbos	5	5.43	4.39	17.50	17.15	0.70	23.75	13.75	55.35
24 Jun	Fynbos	40	5.31	4.69	5.72	17.45	0.85	24.05	12.75	55.10
24 Jun	Fynbos	80	5.64	4.82	4.73	14.25	0.00	6.85	11.00	32.10
24 Jun	Acacia	5	5.38	4.70	52.70	16.35	1.20	78.10	22.15	117.80
24 Jun	Acacia	40	5.67	4.26	22.30	23.15	0.00	14.45	12.45	50.05
24 Jun	Acacia	80	5.16	4.37	26.90	24.05	0.00	6.40	11.05	41.50
24 Jun	Cleared	5	5.24	4.53	85.10	32.15	0.00	147.40	14.20	193.75

Sample details			pH		Ec	Anions (mg/kg)				
Date	Site	Depth	water	KCl	µS/cm	Cl	NO_2^-	NO_3^-	SO_4^{2-}	∑
24 Jun	Cleared	40	5.51	4.60	12.01	22.25	0.00	18.95	11.60	52.80
24 Jun	Cleared	80	5.56	4.72	9.16	16.70	0.00	14.50	11.90	43.10
15 Jul	Fynbos	5	4.96	4.22	6.58	4.40	0.00	5.75	2.45	12.60
15 Jul	Fynbos	40	5.15	4.54	5.58	3.50	0.00	1.40	1.65	6.55
15 Jul	Fynbos	80	5.63	4.80	7.64	3.90	0.00	2.00	2.60	8.50
15 Jul	Acacia	5	5.52	4.61	43.00	16.85	0.50	114.05	14.55	145.95
15 Jul	Acacia	40	5.39	4.22	18.59	18.45	0.00	30.15	11.60	60.20
15 Jul	Acacia	80	5.38	4.56	8.32	20.70	0.00	12.90	13.20	46.80
15 Jul	Cleared	5	6.25	5.62	30.80	9.65	0.00	28.70	5.35	43.70
15 Jul	Cleared	40	5.29	4.54	17.08	9.75	0.20	11.10	4.55	25.60
15 Jul	Cleared	80	7.00	4.81	11.68	9.10	0.00	8.40	5.55	23.05
29 Jul	Fynbos	5	5.72	4.48	13.07	16.05	0.70	17.60	11.85	46.20
29 Jul	Fynbos	40	5.82	4.84	6.95	15.15	0.00	9.45	14.45	39.05
29 Jul	Fynbos	80	5.93	4.81	6.26	13.15	0.00	6.65	11.70	31.50
29 Jul	Acacia	5	5.34	4.73	40.20	13.75	0.50	91.10	12.95	118.30
29 Jul	Acacia	40	5.27	4.45	8.70	17.20	0.00	7.25	7.00	31.45
29 Jul	Acacia	80	4.95	4.50	6.01	17.55	0.00	5.10	6.90	29.55
29 Jul	Cleared	5	5.70	4.74	8.57	4.85	0.35	13.10	3.65	21.95
29 Jul	Cleared	40	4.98	4.50	45.68	3.05	0.00	9.20	2.50	14.75
29 Jul	Cleared	80	5.26	4.59	4.53	1.95	0.00	2.45	1.60	6.00
10 Aug	Fynbos	5	5.51	4.82	7.79	3.60	0.00	8.90	3.15	15.65
10 Aug	Fynbos	40	5.61	4.24	8.62	4.05	0.00	3.30	2.80	10.15
10 Aug	Fynbos	80	6.31	4.61	4.47	5.65	0.00	2.50	2.30	10.45
10 Aug	Acacia	5	5.56	4.25	32.40	5.55	0.00	64.20	5.25	75.00
10 Aug	Acacia	40	5.53	4.56	16.60	6.00	0.00	27.80	3.75	37.55
10 Aug	Acacia	80	6.04	4.63	15.30	5.41	0.04	12.04	3.01	20.50
10 Aug	Cleared	5	6.05	4.65	14.84	3.71	0.00	7.12	3.33	14.16
10 Aug	Cleared	40	5.71	4.77	8.16	3.39	0.00	3.23	3.15	9.77
10 Aug	Cleared	80	5.73	5.11	4.62	3.53	0.22	2.42	3.19	9.36
1 Sep	Fynbos	5	5.22	4.26	10.23	2.70	0.00	10.50	2.25	15.45
1 Sep	Fynbos	40	5.50	4.82	5.53	1.95	0.00	4.30	1.95	8.20
1 Sep	Fynbos	80	5.39	4.63	9.65	19.05	0.00	1.95	4.40	25.40
1 Sep	Acacia	5	5.40	4.62	20.10	3.65	0.00	27.20	3.20	34.05
1 Sep	Acacia	40	5.16	4.55	23.90	9.25	0.00	7.75	3.85	20.85
1 Sep	Acacia	80	5.75	4.50	6.91	3.85	0.00	2.00	1.40	7.25

Sample details			pH		Ec	Anions (mg/kg)				
Date	Site	Depth	water	KCl	µS/cm	Cl	NO_2^-	NO_3^-	SO_4^{2-}	∑
1 Sep	Cleared	5	5.71	4.44	19.44	2.30	0.00	15.35	1.80	19.45
1 Sep	Cleared	40	5.51	4.79	9.84	1.55	0.00	5.90	1.75	9.20
1 Sep	Cleared	80	5.41	4.66	7.92	2.05	0.00	3.90	1.55	7.50
14 Sep	Fynbos	5	5.40	4.76	11.75	4.40	0.20	3.65	2.30	10.55
14 Sep	Fynbos	40	5.16	4.39	6.25	22.95	0.00	9.45	16.60	49.00
14 Sep	Fynbos	80	5.67	4.67	8.49	3.45	0.00	1.00	2.55	7.00
14 Sep	Acacia	5	5.53	4.70	35.20	5.50	0.30	19.95	2.75	28.50
14 Sep	Acacia	40	5.65	4.49	26.90	5.30	0.00	7.35	2.15	14.80
14 Sep	Acacia	80	5.64	4.36	20.10	4.70	0.25	5.00	1.25	11.20
14 Sep	Cleared	5	5.75	4.75	23.90	1.85	0.00	25.80	2.20	29.85
14 Sep	Cleared	40	5.34	4.72	10.53	2.15	0.00	4.60	2.00	8.75
14 Sep	Cleared	80	6.00	4.55	7.79	2.35	0.00	3.70	1.80	7.85
28 Sep	Fynbos	5	5.52	4.58	12.63	27.00	0.00	8.40	15.20	50.60
28 Sep	Fynbos	40	5.98	4.36	13.93	4.24	0.00	25.90	3.19	33.33
28 Sep	Fynbos	80	6.22	4.68	18.28	5.33	0.00	26.56	3.88	35.77
28 Sep	Acacia	5	6.17	4.66	11.14	3.31	0.12	20.40	2.82	26.65
28 Sep	Acacia	40	5.87	4.82	8.10	3.05	0.00	46.80	0.00	49.85
28 Sep	Acacia	80	5.48	4.90	7.90	2.80	0.00	21.60	38.65	63.05
28 Sep	Cleared	5	5.92	4.60	12.71	8.30	0.30	77.50	41.70	127.80
28 Sep	Cleared	40	5.60	4.68	9.68	3.91	0.13	9.36	3.27	16.67
28 Sep	Cleared	80	6.11	4.70	8.76	3.80	0.00	59.45	58.30	121.55
10 Oct	Fynbos	5	5.30	4.39	13.08	3.83	0.00	2.27	3.43	9.53
10 Oct	Fynbos	40	5.32	4.29	9.34	3.55	0.09	2.00	3.19	8.83
10 Oct	Fynbos	80	5.74	4.35	28.00	8.45	0.00	1.98	4.40	14.83
10 Oct	Acacia	5	5.33	4.61	20.00	6.65	0.40	2.85	2.10	12.00
10 Oct	Acacia	40	5.06	4.41	15.09	5.65	0.00	3.90	1.80	11.35
10 Oct	Acacia	80	5.88	4.24	12.44	10.55	0.00	2.35	1.55	14.45
10 Oct	Cleared	5	5.71	4.41	16.65	5.40	0.20	5.15	2.35	13.10
10 Oct	Cleared	40	5.47	4.75	9.80	3.75	0.00	4.65	1.70	10.10
10 Oct	Cleared	80	5.11	4.39	7.90	1.95	1.90	2.15	1.85	7.85
27 Oct	Fynbos	5	5.87	4.89	9.59	7.50	0.00	1.00	3.85	12.35
27 Oct	Fynbos	40	5.75	4.52	8.79	14.40	0.00	0.85	3.70	18.95
27 Oct	Fynbos	80	6.09	4.49	9.98	8.75	0.00	0.00	4.05	12.80
27 Oct	Acacia	5	5.76	4.69	36.80	5.20	0.50	3.10	2.10	10.90
27 Oct	Acacia	40	5.81	4.41	15.25	6.45	0.30	3.70	1.70	12.15

Sample details			pH		Ec	Anions (mg/kg)				
Date	Site	Depth	water	KCl	µS/cm	Cl	NO_2^-	NO_3^-	SO_4^{2-}	∑
27 Oct	Acacia	80	5.93	4.41	10.53	4.50	0.00	2.30	1.15	7.95
27 Oct	Cleared	5	5.91	4.85	15.02	3.45	0.35	2.80	1.75	8.35
27 Oct	Cleared	40	5.95	4.98	10.21	3.30	0.40	2.70	1.30	7.70
27 Oct	Cleared	80	5.92	4.81	6.85	2.35	0.00	2.35	1.15	5.85
9 Nov	Fynbos	5	5.84	4.89	15.61	4.20	0.20	3.45	2.05	9.90
9 Nov	Fynbos	40	5.37	4.75	7.58	3.65	0.00	4.75	2.55	10.95
9 Nov	Fynbos	80	5.98	4.64	14.44	5.60	0.00	0.70	2.30	8.60
9 Nov	Acacia	5	5.81	4.73	13.12	5.80	0.30	6.85	1.95	14.90
9 Nov	Acacia	40	5.57	4.69	9.75	4.10	0.00	5.40	1.80	11.30
9 Nov	Acacia	80	5.59	4.66	12.87	5.10	0.00	2.05	1.40	8.55
9 Nov	Cleared	5	6.03	4.67	16.38	5.30	0.45	9.25	2.10	17.10
9 Nov	Cleared	40	5.72	4.77	25.10	3.00	0.25	13.15	2.30	18.70
9 Nov	Cleared	80	5.58	4.66	17.26	2.80	0.00	5.65	1.60	10.05
9 Dec	Fynbos	5	5.91	4.91	8.42	11.85	0.00	5.40	9.85	27.10
9 Dec	Fynbos	40	5.91	4.19	5.10	15.35	0.00	5.45	11.40	32.20
9 Dec	Fynbos	80	5.83	4.43	6.18	15.30	0.00	4.30	9.65	29.25
9 Dec	Acacia	5	5.26	4.57	5.80	11.95	0.50	10.95	10.55	33.95
9 Dec	Acacia	40	5.44	4.61	8.53	14.55	0.20	8.65	10.65	34.05
9 Dec	Acacia	80	5.59	4.54	9.02	13.00	0.00	5.60	0.00	18.60
9 Dec	Cleared	5	5.70	4.55	10.86	13.60	0.30	11.60	10.25	35.75
9 Dec	Cleared	40	5.99	4.62	11.15	10.00	0.00	9.60	7.70	27.30
9 Dec	Cleared	80	6.02	4.81	10.79	10.80	0.00	7.80	9.15	27.75

Appendix D – Spatial distribution of N concentration in groundwater (mg/l) calculated with Visual MODFLOW (Jovanovic et al., 2008).